IN THE UNITED STATES DISTRICT COURT
FOR THE DISTRICT OF DELAWARE

PFIZER INC., WYETH LLC, PFIZER PHARMACEUTICALS LLC, PF PRISM C.V. and PFIZER MANUFACTURING HOLDINGS LLC,	)	
Plaintiffs,	)	
v.	)	C.A. No. 14-781 (SLR)
CFT PHARMACEUTICALS LLC,	)	
Defendant.	)	

JOINT APPENDIX IN SUPPORT OF CLAIM CONSTRUCTION BRIEFING

VOLUME 3 OF 4

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August 31, 2015

Clinical Therapeutics/Volume 27, Number 1, 2005

Tigecycline: An Investigational Glycylcycline Antimicrobial with Activity Against Resistant Gram-Positive Organisms

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ABSTRACT

Background: Bacterial resistance to currently available antimicrobials is an increasing concern, particularly among various gram-positive organisms such as drug-resistant pneumococci, methicillin-resistant staphylococci, and drug-resistant enterococci. Tigecycline is an investigational glycylcycline antibiotic that shows promising activity against these resistant gram-positive organisms.

Objective: This paper reviews the pharmacology, pharmacokinetic and pharmacodynamic properties, in vitro and in vivo activity, safety profile, and potential role of tigecycline in the management of gram-positive infections involving resistant microbes.

Methods: Articles included in this review were identified through a search of MEDLINE from 1998 through 2004 using the terms *tigecycline* and *GAR-936*. Abstracts from the Interscience Conference on Antimicrobial Agents and Chemotherapy from 1998 to 2003 were searched using the same terms. The reference lists of identified articles were also reviewed for pertinent publications.

Results: Whereas resistance has developed with many of the earlier tetracycline derivatives, tigecycline appears to have a reduced potential for resistance. Several reports have evaluated the in vitro activity of this agent against a number of organisms. It has exhibited pronounced activity against most gram-positive microbes, including resistant strains (eg, drug-resistant pneumococci, methicillin-resistant staphylococci, resistant enterococci). Tigecycline has also shown useful activity against many clinically important gram-negative microbes. In vivo studies of tigecycline are limited. Only 2 clinical trials have been reported to date, one in patients with complicated skin and skin-structure infections and the other in patients with complicated intra-abdominal infections. In these studies, tigecycline therapy resulted in clinical cures in more than two thirds of evaluable patients. Tigecycline was well tolerated in both studies; nausea and vomiting were the most common adverse events.

Conclusions: Although published clinical trials involving tigecycline are limited and additional trials are needed, preliminary reports on its use in the treatment of gram-positive infections are encouraging. Tigecycline has favorable pharmacokinetic properties and, apart from gastrointestinal adverse events, appears to be well tolerated. (*Clin Ther.* 2005;27:12–22) Copyright © 2005 Excerpta Medica, Inc.

Key words: tigecycline, GAR-936, glycylcycline, gram-positive resistance, pharmacology, efficacy, safety.

INTRODUCTION

Over the past several years, there has been growing concern about the increased prevalence of antimicrobial resistance, particularly among various gram-positive organisms such as drug-resistant pneumococci, methicillin-resistant staphylococci, and drug-resistant enterococci.[1,2] In many situations, there are a limited number of effective antimicrobials for use in these resistant infections. Agents such as linezolid, quinupristin/dalfopristin, and daptomycin have become available for managing drug-resistant gram-positive infections.

Although the tetracycline class of antimicrobials has been in place since the mid-1940s, widespread development of resistance has significantly impaired its clinical utility. Mutational changes involving active efflux mechanisms, ribosomal modifications, or both

Accepted for publication November 12, 2004.
doi:10.1016/j.clinthera.2005.01.007
0149-2918/05/$19.00

have rendered many of the earlier tetracycline derivatives ineffective.[3,4] Structural manipulations of the tetracycline molecule have led to more stable compounds, the glycylcyclines.[4-6] Tigecycline (GAR-936) is a representative glycylcycline agent currently under development and shows promising activity against gram-positive and gram-negative organisms, including resistant gram-positive isolates.

The purpose of this paper was to review the pharmacology, pharmacokinetic and pharmacodynamic properties, in vitro and in vivo activity, safety profile, and potential role of tigecycline in the management of gram-positive infections involving resistant microbes.

METHODS

Articles included in this review were identified through a search of MEDLINE from 1998 through 2004 using the terms *tigecycline* and *GAR-936*. Abstracts from the Interscience Conference on Antimicrobial Agents and Chemotherapy from 1998 to 2003 were searched using the same terms. The reference lists of identified articles were also reviewed for pertinent publications.

PHARMACOLOGY AND MECHANISM OF ACTION

The glycylcyclines are semisynthetic analogues of earlier tetracyclines. The chemical structures of tetracycline, doxycycline, minocycline, and tigecycline are illustrated in the figure. The initial glycylcycline compounds had a dimethyl-glycylamido (DMG) group at the C-9 position of the basic tetracycline molecule. These initial compounds—DMG-minocycline and DMG-demethyl-deoxytetracycline—were found to retain the primary antimicrobial activity of the tetracyclines but were less likely to be negatively affected by the primary mechanisms involved in tetracycline resistance: efflux, ribosomal protection, and chemical modification.[3] The development of tigecycline resulted from substitution of a *t*-butylglycylamido group at the C-9 position of minocycline, a modification that resulted in even greater stability against resistance without compromised activity.[3,7] A thorough discussion of the structure–activity relationships of the tetracyclines and glycylcyclines can be found in the recent review by Zhanel et al.[8]

Antimicrobials typically elicit the desired therapeutic response by binding to a specific receptor site with-

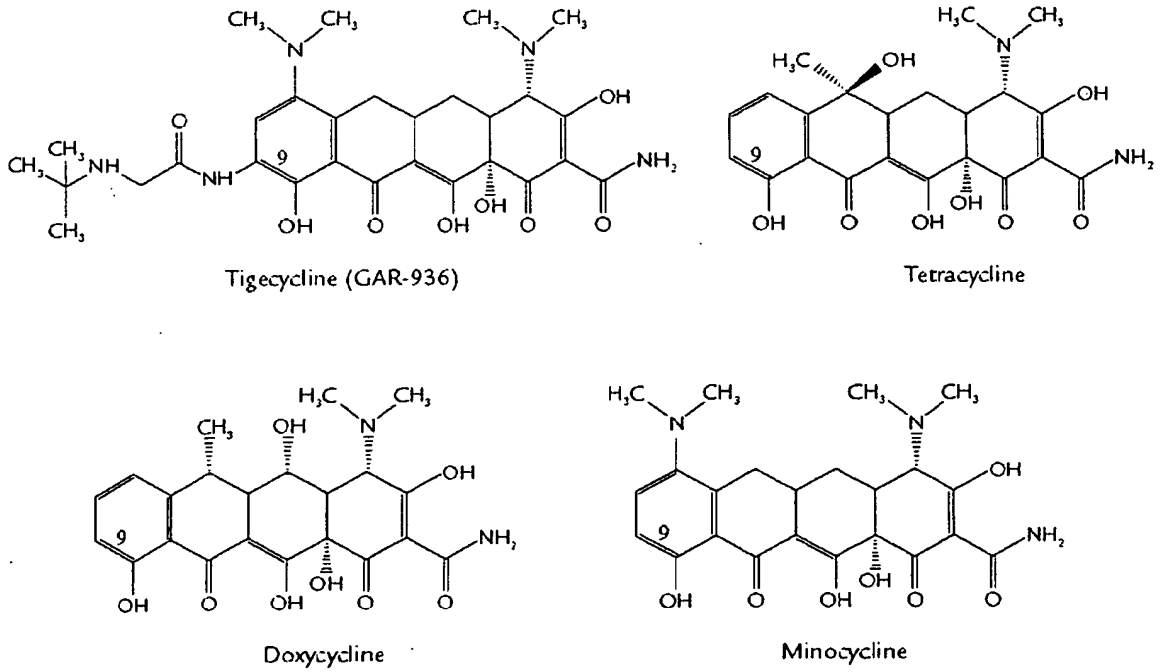

Figure. Chemical structure of the glycylcycline tigecycline (GAR-936) and the related tetracycline agents tetracycline, doxycycline, and minocycline.

in an organism. For tigecycline and the tetracycline class as a whole, the primary receptor site is in the bacterial ribosome. Tigecycline enters the bacterial cell through either energy-dependent pathways or passive diffusion and reversibly binds to the 30S subunit of the ribosome. This reversible binding blocks the crucial incorporation of transfer RNA into the A site of the ribosome and ultimately interferes with protein synthesis.[5] The activity of tigecycline is considered bacteriostatic, probably because of this reversible binding. Compared with tetracycline and minocycline, tigecycline has a considerably stronger binding affinity, making it less likely that strains resistant to tigecycline will emerge rapidly.[4,7]

RESISTANCE

With any new antimicrobial, the possibility of the development of resistance is a significant concern. The primary mechanisms in tetracycline resistance involve either export of the antibiotic from the cell through an active efflux pump or ribosomal protection, which interferes with binding of the antibiotic to the target receptor site. More than 2 dozen tetracycline-resistant, or *tet*, genes have been characterized.[5] Some *tet* genes code for efflux, whereas others influence ribosomal protection. In gram-negative organisms, efflux genes are equally responsible for resistance to tetracycline, minocycline, and doxycycline. In contrast, efflux genes in gram-positive organisms predominantly target tetracycline and have less of an impact on the tetracycline derivatives.[8] As mentioned previously, the unique attribute of tigecycline is its stability against these common mechanisms of tetracycline resistance. Tigecycline has demonstrated activity against strains containing *tet* genes that code for either or both major forms of tetracycline resistance.[4]

The reason for tigecycline's potential to avoid resistance is not entirely clear. Regarding efflux, researchers have hypothesized that tigecycline goes unrecognized by resistant *tet* genes or that the large, bulky side chain at position 9 of tigecycline prohibits the active efflux pump from exporting the compound.[3] In addition, tigecycline and the earlier glycylcyclines have up to 5-fold greater binding affinity for the ribosomal receptor site compared with tetracyclines.[7] This stronger binding affinity of tigecycline may interfere with the *tet* genes' ability to bind effectively to the receptor and protect the ribosome.

Researchers have attempted to create tigecycline-resistant isolates in the laboratory setting using prolonged exposure to suboptimal concentrations of tigecycline. To date, these attempts have been unsuccessful.[4] Furthermore, naturally occurring tigecycline-resistant strains have not been observed in patients.[5] Two veterinary strains of *Salmonella* have been isolated that contained variants of the *tet*(A) gene that conferred resistance to DMG-minocycline and DMG-demethyl-deoxytetracycline.[9] Tigecycline maintained activity against these resistant veterinary strains, demonstrating greater stability and lower resistance potential than the earlier glycylcyclines.[4] Other reports have addressed the possibility of efflux pumps as the cause of reduced susceptibility to tigecycline in *Proteus mirabilis, Pseudomonas aeruginosa*, and *Escherichia coli*.[10–12] Continued monitoring for the emergence of efflux-mediated resistance to tigecycline will be necessary as its development progresses.

PHARMACOKINETICS AND PHARMACODYNAMICS

Tigecycline is currently available only as an investigational parenteral agent. Because of the limited number of clinical trials involving tigecycline, published data on its pharmacokinetic and pharmacodynamic characteristics are also limited. Two independent groups evaluated the pharmacokinetics of tigecycline in a small number of healthy volunteers.[13,14] In one study, 8 male subjects received 7 single IV doses of tigecycline ranging from 12.5 mg to 300 mg, each infused over 1 hour.[13] Dose-proportional C_{max} values were 0.11 and 2.8 μg/mL for the lowest and highest doses studied, respectively, and the corresponding AUCs were 0.9 and 17.9 μg·h/mL. Food increased the maximum tolerated dose of tigecycline from 100 mg (fasting) to 200 mg but did not alter its pharmacokinetics. The mean $t_{1/2}$ was 36 hours. Tigecycline was widely distributed into the tissues, with a steady-state volume of distribution (V_{ss}) of >10 L/kg. It is not known whether tigecycline has an active metabolite.

Similar results were observed in a study in 8 Japanese men who received single-dose 1-hour infusions of tigecycline 25, 50, 100, and 150 mg.[14] Tigecycline was administered shortly after subjects had been fed. Again, C_{max} and AUC values were dose proportional, ranging from 0.2 to 1.5 μg/mL and 0.8 to 8.6 μg·h/mL, respectively. However, the maximum tolerated dose was 100 mg, even though tigecycline

was administered with subjects in the fed state. Additional pharmacokinetic parameters included dose-dependent values for $t_{1/2}$ (range, 8.2–35.5 hours) and V_{ss} (range, 4.4–10.8 L/kg). Total body clearance was 0.3 to 0.5 L/h per kg, and roughly 15% of the tigecycline dose was recovered unchanged in the urine.[14]

The recent clinical trial by Postier et al[15] characterized the steady-state pharmacokinetics of 2 parenteral tigecycline regimens in hospitalized patients with complicated skin or skin-structure infections. In patients receiving the higher tigecycline dose (50 mg IV q12h), mean values for C_{max}, AUC_{0-12}, and clearance were 0.4 μg/mL, 2.24 μg·h/mL, and 0.31 L/h per kg, respectively. In patients receiving the lower tigecycline dose (25 mg IV q12h), the respective values were 0.26 μg/mL, 1.43 μg·h/mL, and 0.25 L/h per kg.

The impact of renal disease on the pharmacokinetics of parenteral tigecycline has also been investigated. After IV administration of a 100-mg dose over 1 hour, the pharmacokinetics of tigecycline were evaluated in 6 healthy subjects, 6 patients with renal impairment (creatinine clearance <30 mL/min), and 8 patients with end-stage renal disease (ESRD) on hemodialysis.[16] Half of the patients with ESRD received tigecycline 2 hours before dialysis, and the remainder received tigecycline after dialysis. Patients with impaired renal function had the same C_{max} as healthy subjects (0.6 μg/mL) but a 40% increase in AUC (4.76 vs 3.33 μg·h/mL, respectively). The mean tigecycline C_{max} was 60% higher in those on dialysis compared with healthy subjects (0.96 vs 0.6 μg/mL), but the AUC was only 20% higher (4.04 vs 3.33 μg·h/mL). The results of this study suggest that tigecycline is not significantly removed by hemodialysis and that despite the increased concentrations observed in patients with ESRD, the dose of tigecycline does not require adjustment in patients with renal impairment.

To determine the potential impact of age and sex on tigecycline pharmacokinetics, a single 100-mg IV dose was given over 1 hour to men and women in 3 age groups (<50 years, 50–75 years, and >75 years).[17] The mean C_{max} ranged from 0.85 to 1.0 μg/mL for all patients. The lowest AUC values were observed in men aged <50 years (4.2 μg·h/mL), and the highest values were in men aged >75 years (5.8 μg·h/mL). The AUC for women of all ages was roughly 5.0 μg·h/mL. The authors reported that tigecycline was well tolerated and safe, and that the pharmacokinetics of tigecycline were not influenced by age or sex.

Several studies addressing the pharmacodynamics of tigecycline have been published.[4,18,19] The pharmacodynamics of tigecycline and another investigational glycylcycline, WAY 152,288, were characterized in a murine thigh infection model.[18] After a dose of tigecycline 3 mg/kg IV, the in vivo postantibiotic effect (PAE) was a respective 8.9 hours and 4.9 hours for strains of *Streptococcus pneumoniae* and *E coli*. The authors suggested that maintaining tigecycline concentrations above the minimum inhibitory concentration (MIC) for ≥50% of the dosing interval was the pharmacodynamic parameter that best correlated with efficacy against strains of *S pneumoniae, E coli*, and *Klebsiella pneumoniae*, and that AUC was an important parameter in predicting efficacy.

In a separate study, twice- and once-daily tigecycline regimens were evaluated for activity against vancomycin-resistant enterococci in a rabbit endocarditis model.[19] In contrast to the findings of the previous study, regimens in which tigecycline concentrations fell below the MIC for <50% of the dosing interval were just as effective as those in which concentrations were constantly above the MIC. The explanation for these conflicting data may involve the rather long $t_{1/2}$, extensive distribution, and noticeable PAE of tigecycline. In vitro pharmacodynamic studies have characterized the PAE of tigecycline for *Staphylococcus aureus* and *E coli*.[4] Three strains were evaluated for each organism: 1 tetracycline-susceptible strain and 2 tetracycline-resistant strains. For tetracycline-susceptible strains of *S aureus* and *E coli*, tigecycline PAE values were 4.1 and 2.9 hours, respectively. For the 2 tetracycline-resistant strains, tigecycline PAE values were 3.5 and >3.0 hours for *S aureus* and 2.6 and 1.8 hours for *E coli*. The PAE values for tigecycline were consistently greater than those for minocycline for all strains tested.

SPECTRUM OF ACTIVITY

In Vitro Activity

Tigecycline demonstrates in vitro activity against most gram-positive and gram-negative aerobes, as well as anaerobes and atypical organisms. The table lists the minimum concentrations required to inhibit 50% and 90% of clinically encountered bacteria (MIC_{50} and MIC_{90}, respectively), as well as MIC ranges, from published reports.[20–44] Provisional MIC breakpoints for susceptibility to tigecycline have been suggested: isolates with MIC values ≤2 μg/mL or zone diameters

Table. In vitro susceptibility data for tigecycline against selected gram-positive, gram-negative, anaerobic, and atypical organisms.

Organism (No. of Isolates)	MIC_{50}, µg/mL	MIC_{90}, µg/mL	MIC Range, µg/mL	References
Gram-positive organisms				
Bacillus anthracis (2)	NA	NA	≤0.03-0.5	36
Corynebacterium jeikeium (41)	0.06	0.125	≤0.015-1.0	20,21,24
JK Diphtheroids (20)	0.5	2.0	0.12-4.0	23
Enterococcus avium (10)	0.06	0.06	0.06-0.12	23
Enterococcus casseliflavus (35)	0.12	0.12	≤0.03-0.5	23,32
Enterococcus faecalis (281)	0.12	0.25	≤0.015-1.0	20,23,24,26-28,30
Glycopeptide resistant (85)	0.12	0.25	≤0.03-1.0	23,24,27,32
Enterococcus faecium (272)	0.125	0.25	0.03-0.25	20,23,24,26-28,30
Glycopeptide resistant (145)	0.12	0.12	≤0.03-0.5	23,24,27,28,32
Enterococcus gallinarum (21)	0.12	0.25	0.06-2.0	23,32
Enterococcus raffinosus (10)	0.06	0.12	0.06-0.5	23
Lactobacillus species (12)	0.06	0.12	0.03-0.12	23
Leuconostoc species (10)	0.12	0.12	0.12	23
Listeria monocytogenes (20)	0.25	0.5	0.25-0.5	23
Staphylococci, coagulase negative (403)	0.25	0.5	0.015-2.0	20,21,24,26,30,31
Methicillin susceptible (70)	0.25	0.5	0.06-1.0	23,24,27,28
Methicillin resistant (124)	0.5	1.0	0.03-2.0	23,24,27,28,32
Staphylococcus aureus (281)	0.06	0.25	≤0.015-1.0	20-22
Methicillin susceptible (602)	0.12	0.25	0.06-0.5	23-28
Methicillin resistant (492)	0.25	0.5	0.06-2.0	23-30
Glycopeptide nonsusceptible (32)	0.25	0.5	0.06-2.0	22,23,27
Streptococcus agalactiae (177)	0.06	0.12	0.015-0.5	23,24,26,28,35
Streptococcus pneumoniae (114)	≤0.015	≤0.015	≤0.015-0.03	20
Penicillin susceptible (5868)	0.03	0.03	0.008-1.0	23,24,26-28,33,34
Penicillin intermediate (1226)	0.03	0.06	≤0.015-1.0	24,26,27,30,33,34
Penicillin resistant (609)	0.03	0.125	≤0.015-1.0	23,24,26-28,30,33,34
Streptococcus pyogenes (318)	0.03	0.06	0.015-0.5	23,24,26,28,35
Streptococcus viridans (239)	0.03	0.12	0.008-2.0	20,24,26
Penicillin susceptible (15)	0.06	0.25	0.03-0.25	23
Penicillin resistant (15)	0.03	0.06	0.02-0.12	23
Gram-negative organisms				
Acinetobacter species (739)	0.5	2.0	0.03-16	24,26,30,37
Burkholderia cepacia (30)	4.0	32	0.5-64	26,28
Citrobacter species (133)	0.5	2.0	0.25-16	24,26,28,30
Eikenella corrodens (108)	0.5	2.0	≤0.06-4.0	21,38
Enterobacter aerogenes (89)	1.0	2.0	0.25-8.0	24,26,28,30
Enterobacter cloacae (144)	1.0	2.0	0.25-4.0	24,26,28,30
Escherichia coli (378)	0.25	0.5	0.06-2.0	20,24,26,28,30
Haemophilus influenzae (118)	0.5	1.0	0.12-2.0	26,28
Beta-lactamase negative (5899)	4.0	4.0	≤0.06-≥8.0	24,39
Beta-lactamase positive (1732)	2.0	4.0	≤0.0-≥4.0	24,39
Hafnia alvei (10)	0.5	2.0	0.25-2.0	24
Klebsiella pneumoniae (261)	0.5	1.0	0.06-16	20,24,26,28,30
Moraxella catarrhalis (2449)	0.25	0.5	≤0.03-4.0	24,26,28,39
Morganella morganii (44)	2.0	4.0	1.0-8.0	24,26

(continued)

Table. (Continued)

Organism (No. of Isolates)	MIC_{50}, µg/mL	MIC_{90}, µg/mL	MIC Range, µg/mL	References
Neisseria meningitides (13)	0.03	0.12	0.015-0.12	24
Pasteurella species (62)	0.06	0.06	0.03-0.125	21
Proteus mirabilis (123)	4.0	8.0	1.0-≥8.0	24,26,28
Proteus vulgaris (55)	4.0	4.0	0.12-16	24,26,28
Providencia species (30)	4.0	8.0	1.0-8.0	24,28
Pseudomonas aeruginosa (145)	8.0	16	0.5-32	24,26,28,30
Salmonella species (34)	0.5	0.5	0.25-2.0	24,28
Serratia marcescens (141)	2.0	4.0	0.5-8.0	24,26,28,30
Shigella species (46)	0.25	0.25	0.12-0.5	24,28
Stenotrophomonas maltophilia (316)	1.0	4.0	0.25-8.0	24,26,28,30,40
Yersinia enterocolitica (10)	0.25	0.5	0.12-0.5	24
Anaerobic organisms				
Bacteroides fragilis (426)	2.0	8.0	≤0.06-32	24,28,30,41
Clostridium difficile (96)	0.06	0.125	≤0.06-2.0	24,28,30
Fusobacterium species (30)	0.06	0.06	≤0.015-0.25	21
Peptostreptococcus species (21)	0.06	0.125	≤0.015-0.5	21
Atypical organisms				
Chlamydia pneumoniae (10)	0.125	0.125	0.125-0.25	42
Legionella species (101)	4.0	8.0	0.5-8.0	43
Mycoplasma hominis (37)	0.25	0.5	0.125-0.5	44
Mycoplasma pneumoniae (30)	0.12	0.25	0.06-0.25	44
Ureaplasma urealyticum (25)	4.0	8.0	1.0-16	44

MIC_{50} = minimum concentration required to inhibit 50% of organisms; MIC_{90} = minimum concentration required to inhibit 90% of organisms; NA = not available.

≥20 mm (using a 30-µg disc) are considered susceptible, and those with MICs ≥8 µg/mL or zone diameters ≤16 mm are considered resistant.[37,45,46]

Tigecycline shows pronounced activity against most gram-positive aerobes. It generally appears equally active or, in many situations, more active than tetracycline and doxycycline against gram-positive organisms.[23] Perhaps the most potentially significant characteristic of tigecycline is its activity against resistant gram-positive bacteria such as penicillin-resistant *S pneumoniae*, methicillin-resistant *S aureus*, and glycopeptide-resistant enterococci. Relevant MIC_{90} values for tigecycline against the foregoing organisms are typically ≤0.5 µg/mL, far lower than those for other tetracycline agents. Even in studies of glycopeptide-resistant enterococci and strains of staphylococci with reduced susceptibility to glycopeptides, the MIC_{90} for tigecycline remained low.[22-24,27-29,32,47]

The activity of tigecycline against *S pneumoniae* appears to be independent of any beta-lactam resistance that might be present. Similar MIC_{50} values are seen against susceptible and nonsusceptible strains of *S pneumoniae*. Although an upward trend in MIC_{90} values has been observed, the value of 0.125 µg/mL for penicillin-resistant *S pneumoniae* is well within the range of susceptibility for tigecycline. Even in strains of streptococci with known resistance to tetracycline, few differences in tigecycline MIC_{50} and MIC_{90} values have been observed between tetracycline-susceptible and tetracycline-resistant strains. This is in sharp contrast to the results for minocycline, which have shown up to a 32-fold increase in MIC for the tetracycline-resistant strains of streptococci tested.[20,45]

Other less common gram-positive organisms, such as *Corynebacterium, Lactobacillus, Leuconostoc*, and *Listeria monocytogenes*, are also susceptible to tigecycline.[20,23,24] Although the data are limited, tigecycline had favorable activity against 2 isolates of *Bacillus anthracis* in preliminary reports.[36]

Tigecycline's activity is not as pronounced against aerobic gram-negative bacteria as against gram-positive organisms. It has activity against *E coli, Haemophilus influenzae, K pneumoniae, Moraxella catarrhalis, Neisseria, Salmonella, Shigella,* and *Yersinia enterocolitica*. The MIC_{90} values for tigecycline approach the proposed susceptibility breakpoints for several other gram-negative organisms, including *Acinetobacter, Citrobacter, Enterobacter,* and *Morganella morganii*. In addition, tigecycline appears to have activity against *Eikenella corrodens, Pasteurella,* and non-*Bacteroides* anaerobes, organisms frequently associated with animal and human bites.[10] Tigecycline has limited or no activity against strains of *Burkholderia cepacia, P mirabilis, Providencia, P aeruginosa, Serratia marcescens,* and *Stenotrophomonas maltophilia*.

Regarding atypical organisms, tigecycline displays pronounced activity against *Mycoplasma hominis, Mycoplasma pneumoniae,* and *Chlamydia pneumoniae*; however, its activity against *Legionella pneumophila* and *Ureaplasma urealyticum* is limited.[42–44] Wallace et al[48] evaluated the activity of tigecycline against several nontuberculous mycobacteria and reported activity against rapidly growing species, such as *Mycobacterium abscessus, Mycobacterium chelonae,* and *Mycobacterium fortuitum*. No activity was observed against slower-growing mycobacterial species (*Mycobacterium avium* complex, *Mycobacterium kansasii, Mycobacterium marinum,* and *Mycobacterium lentiflavum*). Additional evaluation of tigecycline in the clinical setting is needed to fully characterize this agent's specific activity and its potential role in managing infections.

In Vivo Activity

Most in vivo studies to date have involved animal models or small trials in healthy volunteers directed at characterizing the pharmacokinetics of tigecycline. The in vivo animal studies are described briefly here; a more thorough description can be found in the review by Zhanel et al.[8] Trials in an intraperitoneal murine model have assessed the efficacy of tigecycline and daptomycin against methicillin-susceptible, methicillin-resistant, and glycopeptide-intermediate strains of *S aureus*[27]; of tigecycline and minocycline against strains of *S aureus, S pneumoniae,* and *E coli*, including tetracycline-susceptible and tetracycline-resistant isolates[28]; and of tigecycline and several comparators against strains of *Enterococcus faecalis* and *Enterococcus faecium*, again including strains with tetracycline or vancomycin resistance.[49] Additional in vivo murine studies included evaluations of tigecycline activity either alone or in combination with gentamicin or piperacillin in *P aeruginosa* pneumonia[50] and in a neutropenic thigh infection involving strains of *S pneumoniae, S aureus, E coli,* and *K pneumoniae*.[18] Rat and rabbit models of endocarditis have been used to assess the activity of tigecycline against infections involving glycopeptide-susceptible and glycopeptide-resistant enterococci, as well as methicillin-resistant *S aureus*.[51,52] Edelstein et al[43] evaluated the in vivo activity of tigecycline in a guinea pig pneumonia model involving *L pneumophila*. The results of these in vivo animal studies were consistently favorable with respect to the activity of tigecycline against the organisms tested.

A review of the literature identified only 2 Phase II clinical trials involving tigecycline. A multicenter, open-label trial by Postier et al[15] assessed the efficacy and safety profile of 2 tigecycline regimens in hospitalized patients with complicated skin and skin-structure infections. One hundred sixty-four patients were enrolled in the study, but 4 enrollees did not meet the inclusion criteria; thus, the intent-to-treat population included 160 patients who were randomized to receive either tigecycline 50 mg IV q12h with an initial 100-mg IV loading dose (n = 81) or tigecycline 25 mg IV q12h with an initial 50-mg IV loading dose (n = 79). To be considered clinically evaluable, patients had to receive 7 to 14 days of tigecycline therapy, complete a test-of-cure visit within 3 weeks after the initiation of therapy, and receive no concomitant antimicrobial therapy after the first dose of tigecycline. In the 109 clinically evaluable patients, clinical cure rates at the test-of-cure visit were 74% (40/54 patients) for the 50-mg regimen and 67% (37/55 patients) for the 25-mg regimen. Bacteriologic cure rates were 70% (32/46) and 56% (25/45), respectively. The tigecycline MIC_{90} for all pathogens isolated was ≤0.5 μg/mL, and no evidence of resistance to tigecycline was observed. Nausea and vomiting were the most commonly reported adverse events. The authors stated that tigecycline was effective and well tolerated in the management of patients with complicated skin and skin-structure infections.

The second clinical trial, reported in abstract form by Murray et al,[53] evaluated the effectiveness of tige-

cycline in patients with complicated intra-abdominal infections. Patients were included if they had a diagnosis of perforated and gangrenous appendicitis, complicated cholecystitis, perforated diverticulitis, or peritonitis. The study involved 111 patients hospitalized in multiple centers; of these, 15 failed to return and were withdrawn from the study, and only 66 of the remaining patients met the inclusion criteria and were clinically evaluable. All patients received a 100-mg IV loading dose of tigecycline, followed by 50 mg IV q12h for 5 to 14 days. Clinical cure rates at the test-of-cure and end-of-treatment visits in the 66 clinically evaluable patients were 67% (44/66) and 76% (50/66), respectively. Data on bacteriologic cure rates were not available. As in the previous study, nausea and vomiting were the most common adverse events. The authors stated that tigecycline was effective and well tolerated in the treatment of complicated intra-abdominal infections.

SAFETY PROFILE

There is limited information about adverse effects and drug interactions associated with tigecycline therapy. Most of the published data pertain primarily to tetracycline and the tetracycline derivatives. The results of the investigations described in this review suggest that parenteral administration of tigecycline is well tolerated; however, the only report that attempted to characterize the safety profile of tigecycline was the trial by Postier et al.[15]

Consistent with the results of the smaller Phase I studies,[13,14,17] nausea and vomiting were the most common adverse events in the clinical trial by Postier et al.[15] Nausea occurred in 22% (17/79) of patients receiving the low dose of IV tigecycline (25 mg) and in 35% (28/81) of those receiving the high dose (50 mg). Vomiting occurred in 13% (10/79) and 19% (15/81) of the respective dosage groups. Therapy was discontinued as a result of nausea and vomiting in 2 of 160 patients (~1%); both patients had received the high dose of tigecycline. Although more patients receiving the high dose of tigecycline experienced these events, the between-group differences were not found to be significant. Other adverse events included diarrhea, headache, physical pulmonary findings, pain, fever, insomnia, dizziness, hypertension, and anemia. Mild elevations in liver function parameters and blood urea nitrogen were also observed but required no intervention. No life-threatening events occurred during the study. Details of the safety profile of tigecycline were not described in the clinical trial by Murray et al.[53]

In the pharmacokinetic study by Sesoko et al,[14] 1 of 8 healthy subjects receiving a single infusion of tigecycline 100 mg over 1 hour experienced vomiting. When the parenteral dose was increased to a single 150-mg IV dose, 5 of 8 patients experienced nausea and vomiting and another 2 reported nausea only.

The adverse events encountered with tigecycline are similar to those associated with the tetracycline class as a whole.[8] Infusing tigecycline shortly after patients have been fed appears to improve gastrointestinal (GI) tolerability without negatively affecting pharmacokinetics.[13] GI-related adverse drug reactions are perceived to be more common with oral therapies than with IV regimens. However, the data published to date clearly indicate that parenteral administration of tigecycline is associated with nausea and vomiting. The underlying mechanism of this adverse reaction is uncertain. Interestingly, extending the infusion time from 1 to 4 hours had no beneficial effect on tolerability.[13]

CONCLUSIONS

There is a critical need for well-tolerated antimicrobial agents that are effective against the increasing number of resistant organisms. Based on published studies available to date and preliminary information from abstracts, the investigational agent tigecycline has promising activity against many gram-positive organisms, including resistant strains. Tigecycline has shown comparable or better activity than other recently approved agents such as daptomycin. The preliminary data indicate that tigecycline has a low potential for the development of resistance compared with other agents.

The pharmacokinetic profile of tigecycline suggests significant distribution into various tissues and a long half-life, allowing convenient dosing regimens and the possibility of a shorter duration of therapy. An oral formulation of tigecycline is not yet available, and administration is limited to the IV route. This represents a potential disadvantage compared with current agents that are available in both parenteral and oral formulations. Nausea and vomiting are the most common adverse events reported with tigecycline in both healthy volunteers and clinically ill patients.

In general, tigecycline may be an appropriate agent in patients with serious infections caused by resistant

gram-positive organisms. Although early reports are encouraging, ongoing clinical trials involving large numbers of patients will help further define the potential role of tigecycline.

ACKNOWLEDGMENTS

Dr. Garrison has received research grants from Wyeth Pharmaceuticals, and Dr. Setter is a member of the Wyeth Speakers' Bureau.

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ACKNOWLEDGMENTS AND ADDRESSES

Received October 12, 1976, from the *National Center for Antibiotic Analysis, Food and Drug Administration, Washington, DC 20204.*
Accepted for publication January 26, 1977.

Kinetics of Concomitant Degradation of Tetracycline to Epitetracycline, Anhydrotetracycline, and Epianhydrotetracycline in Acid Phosphate Solution

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Abstract □ The concentrations of tetracycline, epitetracycline, anhydrotetracycline, and epianhydrotetracycline in pH 1.5 phosphate solution were followed as a function of time at four temperatures. Separation and quantification of all four species were accomplished using high-pressure liquid chromatography. Through nonlinear regression analysis, rate constants for the reversible first-order epimerization of tetracycline and anhydrotetracycline and for the first-order dehydration of tetracycline and epitetracycline were obtained. Solutions to the differential equations obtained through Laplace transforms successfully predict concentrations found experimentally. The energy of activation for each reaction step was calculated and ranged from 15 to 27 kcal/mole. The rate constants for tetracycline and epitetracycline dehydration conform with those of earlier studies that used different experimental methods. The study shows that epimerization of tetracycline and anhydrotetracycline can take place at a low pH.

Keyphrases □ Tetracycline—kinetics of degradation at pH 1.5, effect of temperature □ Degradation kinetics—tetracycline at pH 1.5, effect of temperature □ Antibacterials—tetracycline, kinetics of degradation at pH 1.5, effect of temperature

Tetracycline degradation to toxic epianhydrotetracycline can take place through tetracycline epimerization to epitetracycline (1, 2) followed by dehydration to epianhydrotetracycline (3) or by dehydration of tetracycline to anhydrotetracycline (4) followed by epimerization to epianhydrotetracycline (5). The kinetics of each individual step have been studied separately under conditions where it was assumed that only the reaction of interest was operative. No reported studies followed all potential reactions simultaneously under the same experimental conditions. The use of high-pressure liquid chromatography (HPLC), which separates all four compounds (tetracycline, epitetracycline, anhydrotetracycline, and epianhydrotetracycline), permits such a study (5). This paper presents the results for the solution degradation of tetracycline at pH 1.5 together with the rate expressions defining these results.

EXPERIMENTAL

Materials—Tetracycline hydrochloride[1] (I), 4-epitetracycline ammonium salt[2] (II), anhydrotetracycline hydrochloride[3] (III), and 4-epianhydrotetracycline[4] (IV) were used as obtained. All other chemicals were reagent grade, and double-distilled deionized water was used to make all solutions.

Apparatus—A high-pressure liquid chromatograph[5] with a multiwavelength detector[6] was used with a 1-m × 2.1-mm strong cation-exchange column[7].

Separation and Quantification—The mobile phase employed in the HPLC separation consisted of 0.07 *M* phosphate–0.0075 *M* ethylenediaminetetraacetic acid adjusted to pH 7.0. To improve the separation for tetracycline and its degradation products, the operating procedure was a slight modification of a previously reported method (5). Specifically, the elution was carried out at a column temperature of 36° and a flow rate of 0.55 ml/min (575 psi). The eluent was monitored at 254 nm at 0.08 absorbance unit full scale (aufs).

Areas under the individual peaks were measured with a polar compensating planimeter[8]. Known injected amounts of I–IV in 0.03 *N* HCl were correlated with the areas under the chromatograms obtained. The slopes of the linear relationship between moles added and area were 2.625×10^{-10}, 2.681×10^{-10}, 1.154×10^{-10}, and 1.377×10^{-10} mole/cm² for I, II, III, and IV, respectively.

Kinetic Method—A phosphoric acid stock solution (1 *M*) was adjusted to pH 1.5 with a concentrated potassium hydroxide solution. The solution was scrubbed with nitrogen and allowed to equilibrate at the temperature desired. Appropriate amounts of I were weighed into volumetric flasks and dissolved in the phosphate solution. The pH did not change during the study. The reaction flasks, sealed with a rubber septum, were immediately placed in a water bath[9] that also protected the solution from light.

At appropriate time intervals, samples were withdrawn, placed in vials, and immersed in ice to stop the reaction. A fixed volume of this sample was assayed chromatographically by equating areas found with concentration through a standard curve. The reactions were followed until all I was lost.

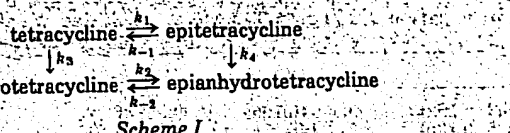
RESULTS AND DISCUSSION

A modification of a previously reported HPLC assay for tetracycline and its degradation products (5) results in less overlap between I and its epimer (Fig. 1). The retention times for IV, III, II, and I were 5.5, 8.25, 22.75, and 30.25 min, respectively.

The relationship between the concentration of each of the four species in the reaction as a function of time gave results as shown in Fig. 2 (obtained at 75°). Four temperatures were used (60–80°), and duplicate kinetic experiments were run at each temperature. Studies made at higher temperatures yielded results involving large errors, making data analysis tenuous.

The concentration–time profile found at each temperature was assumed to be a consequence of the reaction illustrated in Scheme I.

[1] Lot 2K030-71 EA, Pfizer.
[2] Batch 430, British Pharmacopoeia Commission.
[3] Batch 428, British Pharmacopoeia Commission.
[4] Lot 3339-99-1, GS-6659, Pfizer.
[5] DuPont model 830.
[6] DuPont model 835.
[7] DuPont Zipax SCX.
[8] Model 62005, Keuffel and Esser Co.
[9] Haake model FS2.



Scheme I

The rate constants for the individual reaction steps can be obtained from differential equations defined by the model. The equations are:

$$\frac{d[I]}{dt} = k_{-1}[II] - (k_1 + k_3)[I] \quad \text{(Eq. 1)}$$

$$\frac{d[II]}{dt} = k_1[I] - (k_{-1} + k_4)[II] \quad \text{(Eq. 2)}$$

$$\frac{d[III]}{dt} = k_3[I] + k_{-2}[IV] - k_2[III] \quad \text{(Eq. 3)}$$

$$\frac{d[IV]}{dt} = k_2[III] + k_4[II] - k_{-2}[IV] \quad \text{(Eq. 4)}$$

where the rate constants conform to the designation in Scheme I.

The differential equations for I and II (Eqs. 1 and 2) can be solved using Laplace transforms without considering the III and IV reactions since the dehydration process is irreversible first order for both I and II. The solutions to Eqs. 1 and 2 are:

$$[I]_t = \frac{[I]_0(k_{-1} + k_4 - a)}{(b-a)} e^{-at} + \frac{[I]_0(k_{-1} + k_4 - b)}{(a-b)} e^{-bt} \quad \text{(Eq. 5)}$$

$$[II]_t = \frac{[I]_0 k_1}{(b-a)} e^{-at} + \frac{[I]_0 k_1}{(a-b)} e^{-bt} \quad \text{(Eq. 6)}$$

where the subscripts to the concentration terms indicate any time, t, and initial, 0, concentrations and where a and b are complex constants used in the Laplace transforms. They are comprised of the reaction rate constants given in Scheme I and are partly defined in Eqs. 9–11.

When Eqs. 5 and 6 are substituted into Eqs. 3 and 4, the resulting differential equations for III and IV can be solved using Laplace transforms. The integrated equations resulting are:

$$[III]_t = \frac{k_{-2}k_4k_1[I]_0 + k_{-2}k_3(k_{-1} + k_4)[I]_0}{abc} + \frac{k_{-2}k_4k_1[I]_0 + (k_{-2} - a)(k_{-1} + k_4 - a)k_3[I]_0}{(-a)(b-a)(c-a)} e^{-at} + \frac{k_{-2}k_4k_1[I]_0 + (k_{-2} - b)(k_{-1} + k_4 - b)k_3[I]_0}{(-b)(a-b)(c-b)} e^{-bt} + \frac{k_{-2}k_4k_1[I]_0 + (k_{-2} - c)(k_{-1} + k_4 - c)k_3[I]_0}{(-c)(a-c)(b-c)} e^{-ct} \quad \text{(Eq. 7)}$$

and:

$$[IV]_t = \frac{k_2k_4k_1[I]_0 + k_2k_3(k_{-1} + k_4)[I]_0}{abc} + \frac{(k_2 - a)k_4k_1[I]_0 + k_2k_3(k_{-1} + k_4 - a)[I]_0}{(-a)(b-a)(c-a)} e^{-at} + \frac{(k_2 - b)k_4k_1[I]_0 + k_2k_3(k_{-1} + k_4 - b)[I]_0}{(-b)(a-b)(c-b)} e^{-bt} + \frac{(k_2 - c)k_4k_1[I]_0 + k_2k_3(k_{-1} + k_4 - c)[I]_0}{(-c)(a-c)(b-c)} e^{-ct} \quad \text{(Eq. 8)}$$

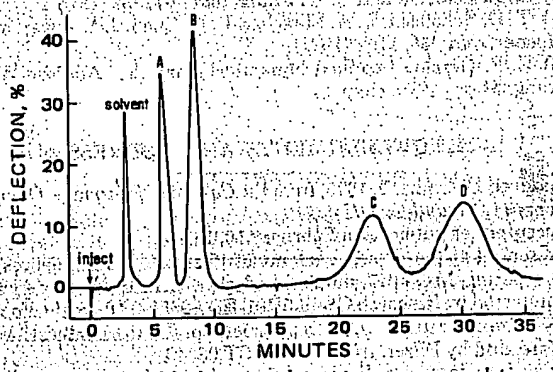


Figure 1—*Relationship between detector response and time in the HPLC separation of IV (A), III (B), II (C), and I (D).*

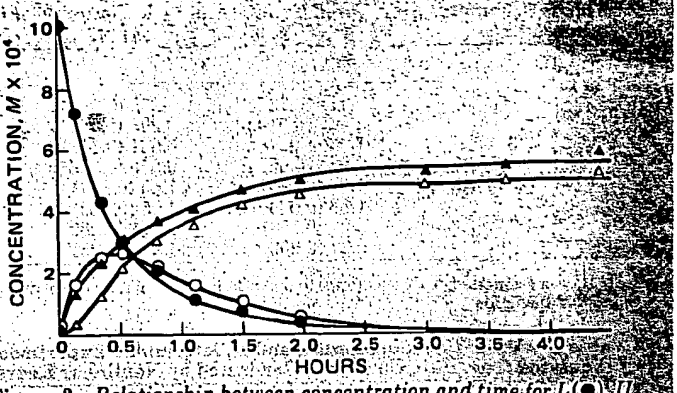


Figure 2—*Relationship between concentration and time for I (●), II (○), III (▲), and IV (△) in pH 1.5 phosphate solution and 75°. The solid lines are the least-squares fit (NONLIN) of the experimental data shown.*

where:

$$a + b = k_1 + k_{-1} + k_3 + k_4 \quad \text{(Eq. 9)}$$

$$ab = k_1k_4 + k_{-1}k_3 + k_3k_4 \quad \text{(Eq. 10)}$$

$$c = k_{-2} + k_2 \quad \text{(Eq. 11)}$$

The experimental data, typified in Fig. 2, were fitted using both the differential (Eqs. 1–4) and integrated (Eqs. 5–8) forms of the equations through nonlinear regression analysis (NONLIN program[10]). To use this program, initial estimates of the six rate constants are required. The values of these initial estimates appear to be quite critical to the outcome of the NONLIN program when using the differential equations (Eqs. 1–4).

A computerized Runge–Kutta method was used to obtain the six initial estimates (6). Trial and error values for the six rate constants defined in Scheme I were used until the Runge–Kutta estimation of the concentrations of I–IV at any time approximately agreed with the concentrations found experimentally. With these Runge–Kutta estimates as initial estimates, the rate constants generated by the NONLIN program were determined (Table I). The largest errors obtained were in the constants for the epimerization of III (k_2 and k_{-2}).

The use of the integrated forms of the equations in the NONLIN program yielded rate constants that were relatively insensitive to the initial estimates. The rate constants generated and their accompanying standard deviations were virtually identical to those found using the differential forms (Table I). For example, when the integrated equations (Eqs. 5–8) were used with the data obtained in Trial 1 at 80°, where the largest errors were found, the rate constants generated (standard deviations in parentheses) were 2.08 (0.153), 0.937 (0.279), 1.87 (0.654), 2.19 (0.729), 1.56 (0.0897), and 2.38 (0.172) for k_1, k_{-1}, k_2, k_{-2}, k_3, and k_4, respectively.

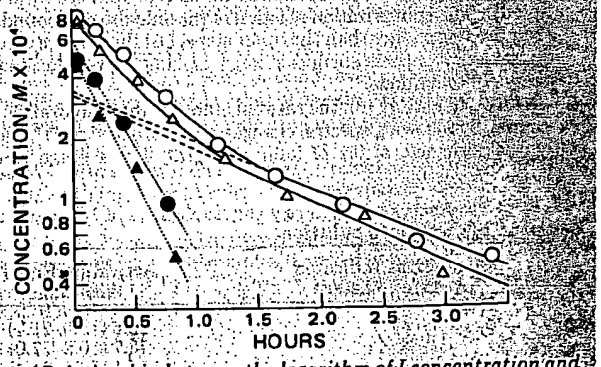


Figure 3—*Relationship between the logarithm of I concentration and time for two trials (○ and △) at 70°. Broken lines (- - -) indicate terminal slope and dotted lines (· · ·) indicate feathered initial data for Trial 1 (●) and Trial 2 (▲).*

[10] Unit 7292, The Upjohn Co.

Table I—Computer-Generated Rate Constants in Hours^{-1} for the Degradation of I in pH 1.5 Phosphate Solution at Several Temperatures

Temperature ± 0.5°	k_1[a]	k_{-1}	k_2	k_{-2}	k_3	k_4
60°[b]	0.414 (0.0274)[c]	0.373 (0.0531)	0.659 (0.162)	0.665 (0.166)	0.323 (0.0207)	0.206 (0.0344)
	0.392 (0.0192)	0.296 (0.0375)	0.585 (0.130)	0.607 (0.135)	0.302 (0.0156)	0.231 (0.0252)
70°	1.03 (0.0978)	0.661 (0.178)	1.31 (0.715)	1.46 (0.780)	0.687 (0.0714)	0.732 (0.118)
	0.982 (0.0504)	0.655 (0.0966)	1.33 (0.300)	1.46 (0.325)	0.743 (0.0369)	0.662 (0.0613)
75°	1.65 (0.0901)	1.43 (0.184)	1.88 (0.422)	2.07 (0.466)	1.28 (0.0677)	0.921 (0.123)
	1.53 (0.0800)	1.05 (0.159)	1.92 (0.436)	2.15 (0.483)	1.23 (0.0601)	1.11 (0.108)
80°	2.08 (0.153)	0.941 (0.282)	1.87 (0.673)	2.19 (0.750)	1.56 (0.0905)	2.37 (0.176)
	2.27 (0.208)	1.58 (0.405)	1.80 (0.909)	2.03 (0.996)	1.60 (0.128)	2.17 (0.251)

[a] Rate constant designation conforms to that of Scheme I. [b] The results of duplicate trials run at each temperature are listed. [c] Parentheses contain the NONLIN-generated standard deviations.

Table II—Values for Constants *a* and *b* Determined Experimentally at Four Temperatures and a Comparison of Their Sum with that Calculated from Rate Constants Generated by Nonlinear Regression Analysis

Temperature	Trial	a, hr^{-1}	b, hr^{-1}	$a+b$ Experimental	$a+b$ Calculated[a]	Ratio of Sums, Calc./Exp.
60°	1	0.91	0.22	1.13	1.32	1.17
	2	1.1	0.22	1.32	1.22	0.92
70°	1	2.4	0.59	2.99	3.11	1.04
	2	2.7	0.63	3.33	3.04	0.91
75°	1	3.8	0.99	4.79	4.81	1.00
	2	3.1	1.0	4.10	4.92	1.20
80°	1[b]	6.4[c]	2.1	8.5	7.8	0.92
	2[d]	6.4	2.1	8.5	7.6	0.89

[a] Using the data from Table I and Eq. 9. [b] Only six data points were available. [c] The 80° data for Trials 1 and 2 were used as one set in the semilogarithmic plots. [d] Only five data points were available.

The constants a and b in Eqs. 5 and 6 also can be obtained from semilogarithmic plots of concentration *versus* time using either I or II data, with the terminal slope reflecting b and the slope of the feathered initial data reflecting a. With the concentration–time data for I, semilogarithmic plots were constructed as represented by those in Fig. 3 for the two trials made at 70°. The a and b values obtained from such plots are given in Table II.

A comparison of the sum of the experimentally obtained a and b constants was made with the sum calculated according to Eq. 9 using the constants (Table I) generated by nonlinear regression analysis. These sums, together with their ratio (calculated to experimental), are given in Table II. The data at 80° are subject to considerable error since the number of data points available to estimate initial and terminal slopes was small due to the fast reaction rates. The agreement between the calculated and experimental sums of a and b is quite good. The average of the ratios (calculated to experimental) is 1.01.

Experimental confirmation of the constant c (Eq. 11) is difficult because it is necessary to construct a plot of the logarithm of the difference in equilibrium and time t concentrations as a function of time (Eq. 7 or 8). The difference in concentrations at long times is subject to large errors, making estimates of the terminal log-linear slope (constant c) highly inaccurate.

An Arrhenius plot of the average rate constant for each reaction step was made using the results obtained at four temperatures (Table I). Estimates of the activation energies for all six reactions are summarized in Table III. The epimerization reaction of the anhydro compounds appears to require less energy than the other steps, but the error involved in the determination of these energies is large, making any conclusion regarding energy effect tenuous.

In an earlier study (3), the rate constant for the dehydration of II, determined by following spectral changes, was 0.618 hr^{-1} at 71° and pH 1.53 (hydrochloric acid at 0.1 M ionic strength). The average value in the present study at 70° was 0.697 hr^{-1}, which is in reasonable agreement with the earlier value found under slightly different conditions. Schlecht and Frank (4) studied I dehydration at various temperatures and hydrogen-ion concentrations. By using their activation energy value (4) of 25.1 kcal/mole (ionic strength of 1 M), it is possible to calculate the rate constant at 60° if it is assumed that the hydrogen-ion concentration is reasonably close to the present hydrogen-ion activity (0.0361 M). Based on their rate constant at 50° (4), the rate constant calculated at 60° is about 0.44 hr^{-1}, which is of the same order of magnitude as the constants found in the present study, 0.323 and 0.302 hr^{-1}, again under different conditions.

In earlier studies on II (3) and I (4) dehydration, it was validly assumed that no epimerization was taking place at pH < 2. However, the present study shows that epimerization of both I and II can occur at pH 1.5, but the system used was different from that of both earlier studies. The present study used a 1 M phosphate solution adjusted to pH 1.5, which should contain significant amounts of monobasic phosphate, a species known to catalyze epimerization (1, 5). The significance of this result is that if a catalytic species is present in sufficient amounts, significant epimerization can occur even if the pH is less than 3.

Table III—Activation Energy for Each Step in I Degradation in pH 1.5 Phosphate Solution

Reaction as Indicated by Rate Constant	Activation Energy, kcal/mole
k_1	20.5 (19.0–21.7)[a]
k_{-1}	18.7 (10.2–27.0)
k_2	14.1 (3.84–24.4)
k_{-2}	15.3 (4.33–26.2)
k_3	19.4)12.8–25.8)
k_4	26.6 (17.3–36.0)

[a] Parentheses contain the upper and lower 95% confidence limits obtained from linear regression analysis.

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ACKNOWLEDGMENTS AND ADDRESSES

Received December 9, 1976, from the *College of Pharmacy, Ohio State University, Columbus, OH 43210.*

Accepted for publication February 10, 1977.

Presented at the APhA Academy of Pharmaceutical Sciences, New York meeting, May 1977, under a Lederle Pharmacy Faculty Award.

Supported by Research Grant 5R01-FD-00689-03 from the Food and Drug Administration, U.S. Department of Health, Education, and Welfare, and by Pfizer, Inc., Groton, CT 06340.

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OLUME 46, NUMBER 8 PUBLISHED MONTHLY AUGUST 2002

ANTIMICROBIAL AGENTS AND CHEMOTHERAPY

AMERICAN SOCIETY FOR MICROBIOLOGY

ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Aug. 2002, p. 2595–2601 Vol. 46, No. 8
0066-4804/02/$04.00+0 DOI: 10.1128/AAC.46.8.2595–2601.2002

In Vitro and In Vivo Activities of Tigecycline (GAR-936), Daptomycin, and Comparative Antimicrobial Agents against Glycopeptide-Intermediate *Staphylococcus aureus* and Other Resistant Gram-Positive Pathogens

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Received 16 January 2002/Returned for modification 28 February 2002/Accepted 1 May 2002

Tigecycline (GAR-936) and daptomycin are potent antibacterial compounds in advanced stages of clinical trials. These novel agents target multiply resistant pathogenic bacteria. Daptomycin is principally active against gram-positive bacteria, while tigecycline has broad-spectrum activity. When tested by the standard protocols of the National Committee for Clinical Laboratory Standards in Mueller-Hinton broth II, tigecycline was more active than daptomycin (MICs at which 90% of isolates tested are inhibited, 0.12 to 1 and 0.5 to 16 μg/ml, respectively) against staphylococcal, enterococcal, and streptococcal pathogens. Daptomycin demonstrated a stepwise increase in activity corresponding to an increase in the supplemental concentration of calcium. When tested in base Mueller-Hinton broth supplemented with 50 mg of calcium per liter, daptomycin demonstrated improved activity (MIC_{90}s, 0.015 to 4 μg/ml). The activity of daptomycin, however, equaled that of tigecycline against the glycopeptide-intermediate *Staphylococcus aureus* (GISA) strains only when the test medium was supplemented with excess calcium (75 mg/liter). Tigecycline and daptomycin demonstrated in vivo efficacies against GISA, methicillin-resistant *S. aureus*, and methicillin-susceptible *S. aureus* strains in an intraperitoneal systemic murine infection model. These data suggest that tigecycline and daptomycin may offer therapeutic options against clinically relevant resistant pathogens for which current alternatives for treatment are limited.

Tigecycline (GAR-936), a glycylcycline (36), and daptomycin, a lipopeptide (1), are novel antibacterial compounds undergoing clinical development. Tigecycline is a broad-spectrum, protein-inhibiting, antibacterial agent possessing activity against strains resistant to other chemotherapeutic agents (14, 29). Daptomycin, a cell wall-inhibiting antibiotic with a spectrum of activity limited to gram-positive bacteria, has also been demonstrated to have activity against resistant bacteria (34). Early clinical trials with daptomycin were discontinued due to less-than-desired outcomes (32) including unwanted side effects on skeletal muscle. However, new dosage regimens (27) have allowed daptomycin to progress into clinical trials (37). These antibacterial agents offer new alternatives for the treatment of infections caused by clinically relevant pathogens for which limited therapeutic options exist.

The rise in the incidence of methicillin-resistant *Staphylococcus aureus* (MRSA) strains (28) and the emergence of strains with intermediate glycopeptide resistance (38) have emphasized the lack of therapeutic alternatives. Recently, a collection of glycopeptide-intermediate *S. aureus* (GISA) strains with reduced susceptibilities to the glycopeptide antibiotics (vancomycin and teicoplanin) has been assembled by the Network on Antibiotic Resistance in *Staphylococcus aureus* (NARSA). That study was undertaken to evaluate the in vitro activities of tigecycline, daptomycin, and comparative antibiotics against these GISA and other drug-resistant gram-positive isolates by the standard methodology of the National Committee for Clinical Laboratory Standards (NCCLS) (26). The activity of daptomycin was determined in both Mueller-Hinton broth II (MHB II) and Mueller-Hinton broth supplemented with 50 mg of calcium per liter. In addition, the effects of calcium concentration and the culture medium on the activities of the antibiotics were determined for the GISA, MRSA, and methicillin-susceptible *S. aureus* (MSSA) isolates, as daptomycin is a calcium-dependent antibiotic. The supplemental calcium concentrations (25, 50, and 75 mg/liter) recommended by other investigators (34) were used for these studies.

MATERIALS AND METHODS

Organisms. Routine clinical isolates were collected from various medical centers in the United States and Canada between 1990 and 1999. Identification of each culture was performed by conventional methodologies. The species of staphylococci were determined with the Staph Trac system (bioMerieux, Hazelwood, Mo.), and confirmation of the species as *S. aureus* was also done by use of a coagulase test. Methicillin resistance in *S. aureus* was determined by growth of the isolate on a Trypticase soy agar plate containing 6 μg of oxacillin per ml plus 2% NaCl (35), and methicillin resistance was confirmed by determination of the oxacillin MICs in the presence of 2% NaCl. The GISA strains were obtained from NARSA (http://narsaweb.narsa.net). Although a vancomycin MIC of 8 to 16 μg/ml defines a GISA strain, not all of the strains in the NARSA collection meet this criteria. All strains were, however, less susceptible to vancomycin than most clinical isolates. The identification of isolates as *Streptococcus pneumoniae* was determined with the API 20 Strep system (bioMerieux). Penicillin-resistant *S. pneumoniae* isolates (MICs, ≥2 μg/ml) were obtained from A. Barry, Clinical Microbiology Institute, Tualatin, Oreg., and S. Block, Bardstown, Ky. Species

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were confirmed to be enterococci by the biochemical tests recommended by Facklam and Collins (10). Strains of vancomycin-resistant enterococci were obtained from the sources described previously (39). All isolates were stored frozen in skim milk plus 50% glycerol at −70°C.

Antibiotics. A standard powder of tigecycline (GAR-936) was obtained at Wyeth-Ayerst Laboratories, Pearl River, N.Y.; daptomycin was obtained from Eli Lilly & Company, Indianapolis, Ind.; teicoplanin was obtained from Marion Merrell Dow Inc., Kansas City, Mo.; vancomycin, erythromycin, and amoxicillin were obtained from Sigma Chemical Co., St. Louis, Mo.; and levofloxacin was obtained from The R. W. Johnson Pharmaceutical Research Institute, Princeton, N.J.

Antimicrobial susceptibility testing. The in vitro activities of the antibiotics were determined by the broth microdilution method recommended by the NCCLS (26). MHB II (BBL, Cockeysville, Md.) was used for the standard NCCLS testing procedures. The label of this cation-adjusted medium states that it contains 20 to 25 mg of calcium per liter. The effects of the calcium concentrations were determined in the following media: base Mueller-Hinton broth (MHB; no calcium supplementation) and MHB supplemented with 25, 50, or 75 mg of calcium per liter (MHB 25, MHB 50, and MHB 75, respectively). Unsupplemented brain heart infusion broth (BHI) and BHI supplemented with 50 mg of calcium per liter (BHI 50) were used for optimal expression of the GISA phenotype (3). The final calcium concentrations in the various supplemented media determined by inductively coupled plasma-optical emission spectrometry (Vista Pro Axial; Varian) were as follows: 20 mg/liter for MHB II, 19.3 mg/liter for MHB, and 37.5, 67.5, and 75 mg/liter for MHB 25, 50, and 75, respectively. The calcium concentrations were 12 mg/liter for BHI and 51 mg/liter for BHI 50. Microtiter plates containing serial dilutions of each antimicrobial agent were inoculated with each organism to yield the appropriate density (10^5 CFU/ml) in a final volume of 100 μl. The plates were incubated for 18 to 22 h at 35°C in ambient air. For all isolates the MIC was defined as the lowest concentration of antimicrobial agent that completely inhibits the growth of the organism as detected by the unaided eye.

In vivo efficacy against murine infections. The therapeutic effects of the antibiotics against acute lethal infections in mice caused by susceptible and resistant *S. aureus* isolates were determined (7). Female strain CD-1 mice (weight, 20 ± 2 g each; Charles River Laboratories, Portage, Mich.) were challenged by intraperitoneal injection of 0.5 ml of a bacterial suspension in hog gastric mucin (10 to 100 median lethal doses). Each antibiotic was administered as a single intravenous dose (0.2 ml) in phosphate-buffered saline (0.01 M; pH 7.4) to five mice per group at 0.5 h postinfection. All of the untreated controls died within 48 h of infection. The median effective dose (ED_{50}) from pooled data obtained from three separate experiments for each organism were determined by probit analysis based on the 7-day survival ratios (11).

RESULTS

The in vitro antibacterial activities of tigecycline, daptomycin, and the comparative antibiotics against resistant and susceptible gram-positive strains determined by the guidelines recommended by the NCCLS with standard MHB II and MHB 50 (for daptomycin) are displayed in Table 1. Tigecycline demonstrated similar in vitro activities against the GISA and the methicillin-resistant and methicillin-susceptible staphylococcal strains tested (MICs at which 90% of isolates tested are inhibited [MIC_{90}s], 0.5 to 1 μg/ml). Against the GISA strains in MHB II, tigecycline was 16 times more active than vancomycin and teicoplanin (MIC_{90}, 8 μg/ml), 32 times more active than daptomycin (MIC_{90}, 16 μg/ml), and at least 64 times more active than levofloxacin, erythromycin, and amoxicillin (MIC_{90}s, 32 to >32 μg/ml). The activities of daptomycin against the GISA strains increased by 2 dilutions when daptomycin was tested in MHB 50 (MIC_{90}, 4 μg/ml); however, it was still 3 dilutions less active than tigecycline. Daptomycin had MIC_{90}s of 1 to 2 μg/ml when it was tested in MHB II but was also 1 to 2 dilutions less active than tigecycline against the glycopeptide-susceptible, methicillin-resistant, and methicillin-susceptible staphylococcal isolates. The in vitro activity of daptomycin increased by 2 dilutions (MIC_{90}s, 0.25 to 0.5 μg/ml) when it was tested in MHB 50 against these same isolates, with its activity equaling or exceeding that of tigecycline. Tigecycline was as active as or more active than vancomycin and teicoplanin (MIC_{90}s, 0.5 to 16 μg/ml) against all of the glycopeptide-susceptible staphylococcal strains tested. Against methicillin-resistant staphylococcal strains, tigecycline was at least 16 times more active than levofloxacin and 32 times more active than erythromycin and amoxcillin.

Tigecycline showed good in vitro activities, with a range of MIC_{90}s of 0.12 to 0.5 μg/ml for vancomycin-susceptible and -resistant strains of *Enterococcus faecalis* and *Enterococcus faecium* (Table 1). The activity of tigecycline was equivalent to that of teicoplanin and slightly greater than that of vancomycin against vancomycin-susceptible isolates (MIC_{90}s, 0.12 to 0.5 and 2 μg/ml, respectively). The activities of tigecycline against vancomycin-resistant enterococcal strains exceeded those of the glycopeptide antibiotics erythromycin and amoxicillin (MIC_{90}s, 16 to >32 μg/ml). Daptomycin was at least 32 times less active than tigecycline against the enterococcal isolates when it was tested in MHB II and was 1 to 4 dilutions less active than tigecycline when it was tested in MHB 50 (MIC_{90}s, 8 to 16 and 1 to 2 μg/ml, respectively). Tigecycline was 2 to 3 dilutions more active than levofloxacin against all *E. faecalis* and vancomycin-susceptible *E. faecium* strains tested (MIC_{90}s, 1 to 2 and 2 μg/ml, respectively). However, the activity of tigecycline against vancomycin-resistant strains of *E. faecium* exceeded that of levofloxacin (MIC_{90}s, >32 μg/ml) by at least 9 dilutions.

The activities of tigecycline against *S. pneumoniae* isolates, including penicillin-resistant, -intermediate, and -sensitive isolates, are shown in Table 1. Tigecycline had MIC_{90}s of 0.25 μg/ml for all of the *S. pneumoniae* strains and demonstrated similar activities against all of the *S. pneumoniae* strains tested. The activities of tigecycline and daptomycin (in MHB II) against penicillin-resistant *S. pneumoniae* isolates were similar to those of vancomycin and levofloxacin (MIC_{90}s, 0.25 to 0.5 μg/ml), but the activities of tigecycline and daptomycin were exceeded by the activities of teicoplanin and daptomycin (in MHB 50) (MIC_{90}s, ≤0.008 and 0.015 μg/ml, respectively). Overall, all of the antibiotics tested demonstrated good activities against the penicillin-intermediate and -susceptible *S. pneumoniae* isolates (MIC_{90} range, 0.015 to 1 μg/ml).

When MHB was supplemented with increased concentrations of calcium, as recommended by Snydman et al. (34), the activities of daptomycin against GISA, MRSA, and MSSA strains were enhanced. This same supplementation of the growth medium with calcium, however, did not alter the activities of tigecycline, vancomycin, or teicoplanin (Table 2). Calcium-supplemented media also had no effect on the activities of levofloxacin, erythromycin, and amoxicillin (data not shown). There was a stepwise increase in the activity of daptomycin, with supplementation with the largest calcium concentration (75 mg/liter) resulting in the greatest increase in activity. Compared to MHB, the activity of daptomycin against the GISA strains increased 16-fold when MHB 75 was used (MIC_{90}s, 16 and 1 μg/ml, respectively). The activity of daptomycin also increased eightfold against MRSA strains (MIC_{90}s, 4 and 0.5 μg/ml) and MSSA strains (MIC_{90}s, 2 and 0.25 μg/ml) when MHB 75 was used. Similar increases in the activities of daptomycin, corresponding to the presence of increased cal-

TABLE 1. In vitro activities of tigecycline, daptomycin, and the comparative antibiotics against recent clinical isolates

Organism (no. of isolates tested) and antibiotic	MIC (μg/ml) Range	50%	90%
S. aureus			
Glycopeptide intermediate (19)			
Tigecycline	0.06–1	0.25	0.5
Daptomycin	2–16	4	16
Daptomycin[a]	0.5–16	1	4
Vancomycin	1–8	4	8
Teicoplanin	0.5–16	4	8
Levofloxacin	0.25–32	16	32
Erythromycin	0.12–>32	>32	>32
Amoxicillin	1–>32	32	>32
Methicillin resistant (10)			
Tigecycline	0.12–1	0.25	0.5
Daptomycin	1–4	2	2
Daptomycin[a]	0.25–0.5	0.25	0.5
Vancomycin	1–2	1	1
Teicoplanin	0.25–8	0.25	1
Levofloxacin	0.06–16	8	8
Erythromycin	>32	>32	>32
Amoxicillin	32–>32	>32	>32
Methicillin susceptible (10)			
Tigecycline	0.25–0.5	0.25	0.5
Daptomycin	1–2	1	2
Daptomycin[a]	0.12–0.5	0.25	0.5
Vancomycin	0.5–1	1	1
Teicoplanin	0.25–1	0.25	0.5
Levofloxacin	0.06–0.12	0.06	0.12
Erythromycin	0.25–>32	0.25	0.5
Amoxicillin	0.25–>32	>32	>32
Coagulase-negative staphylococci			
Methicillin-resistant (10)			
Tigecycline	0.5–2	1	1
Daptomycin	0.5–4	1	2
Daptomycin[a]	0.12–0.5	0.25	0.5
Vancomycin	1–4	2	2
Teicoplanin	2–32	4	16
Levofloxacin	0.12–32	0.25	32
Erythromycin	0.12–>32	32	32
Amoxicillin	4–>32	16	>32
Methicillin susceptible (10)			
Tigecycline	0.5–1	0.5	0.5
Daptomycin	0.5–1	1	1
Daptomycin[a]	0.06–0.5	0.12	0.25
Vancomycin	0.5–2	1	1
Teicoplanin	0.06–2	0.25	1
Levofloxacin	0.06–0.25	0.12	0.25
Erythromycin	0.12–>32	0.25	0.25
Amoxicillin	≤0.03–>32	0.5	8
E. faecalis (10)			
Tigecycline	0.06–0.5	0.12	0.25
Daptomycin	4–16	4	8
Daptomycin[a]	0.12–2	1	1
Vancomycin	1–2	2	2
Teicoplanin	0.06–0.25	0.06	0.12
Levofloxacin	0.5–16	0.5	1
Erythromycin	0.5–>32	2	32
Amoxicillin	0.25–0.5	0.25	0.5
E. faecalis, vancomycin resistant (10)			
Tigecycline	≤0.03–0.5	0.12	0.5
Daptomycin	1–16	8	16
Daptomycin[a]	0.12–2	1	1
Vancomycin	>32	>32	>32
Teicoplanin	0.12–>32	32	>32
Levofloxacin	0.25–32	1	2
Erythromycin	2–>32	>32	>32
Amoxicillin	0.25–16	0.25	16
E. faecium (10)			
Tigecycline	≤0.03–0.25	0.06	0.25
Daptomycin	8–16	16	16
Daptomycin[a]	1–4	1	2
Vancomycin	0.5–2	1	2
Teicoplanin	0.12–0.5	0.12	0.5
Levofloxacin	1–16	1	2
Erythromycin	0.5–>32	4	>32
Amoxicillin	0.12–32	2	16
E. faecium vancomycin resistant (10)			
Tigecycline	0.06–0.25	0.06	0.12
Daptomycin	8–32	16	16
Daptomycin[a]	1–2	2	2
Vancomycin	32–>32	>32	>32
Teicoplanin	0.06–>32	32	>32
Levofloxacin	1–>32	>32	>32
Erythromycin	>32	>32	>32
Amoxicillin	1–32	16	32
Streptococcus pneumoniae			
Penicillin resistant (10)			
Tigecycline	0.25	0.25	0.25
Daptomycin	0.25–1	0.25	0.5
Daptomycin[a]	≤0.008–0.015	≤0.008	0.015
Vancomycin	0.12–0.25	0.25	0.25
Teicoplanin	≤0.008	≤0.008	≤0.008
Levofloxacin	0.5	0.5	0.5
Erythromycin	0.015–4	0.5	4
Amoxicillin	0.5–4	0.5	4
Penicillin intermediate (10)			
Tigecycline	0.12–0.25	0.25	0.25
Daptomycin	0.25–0.5	0.5	0.5
Daptomycin[a]	≤0.008–0.015	≤0.008	0.015
Vancomycin	0.12–0.25	0.25	0.25
Teicoplanin	≤0.008–0.015	≤0.008	0.015
Levofloxacin	0.5–1	1	1
Erythromycin	≤0.008–2	0.015	0.03
Amoxicillin	0.03–0.5	0.12	0.25
Penicillin susceptible (10)			
Tigecycline	0.25–0.5	0.25	0.25
Daptomycin	0.25–4	0.5	0.5
Daptomycin[a]	≤0.008–0.015	≤0.008	0.03
Vancomycin	0.12–0.5	0.25	0.25
Teicoplanin	≤0.008–0.015	≤0.008	0.015
Levofloxacin	0.5–1	0.5	1
Erythromycin	≤0.008–0.03	0.015	0.015
Amoxicillin	≤0.008–0.03	<0.008	0.03

[a] Daptomycin tested in MHB 50.

cium concentrations, were observed against the quality control organisms (Table 3).

The activities of tigecycline against the *S. aureus* strains tested with either MHB II or BHI as the growth medium are shown in Table 2. There was a slight increase in the level of glycopeptide antibiotic resistance when BHI was used as the growth medium for the GISA strains. The range of MICs of vancomycin and teicoplanin increased from 0.5 to 16 μg/ml in MHB II to 2 to 16 μg/ml in BHI. Although this was not a marked shift in the MICs, 47% of the GISA strains showed a

TABLE 2. Effects of calcium and medium on in vitro activities of tigecycline, daptomycin, vancomycin, and teicoplanin

S. aureus phenotype (no. of isolates tested)	Antibiotic	Medium	MIC (μg/ml)		
			Range	50%	90%
Glycopeptide intermediate (19)	Tigecycline	MHB II	0.06–1	0.25	0.5
		MHB	0.06–1	0.12	0.5
		MHB 25	0.06–1	0.12	0.5
		MHB 50	0.06–1	0.12	0.25
		MHB 75	0.06–1	0.12	0.5
		BHI	≤0.03–0.25	≤0.03	0.25
	Daptomycin	MHB II	2–16	4	16
		MHB	4–16	8	16
		MHB 25	0.5–4	2	4
		MHB 50	0.5–16	1	4
		MHB 75	0.25–2	0.5	1
		BHI	>32	>32	>32
		BHI 50	4–16	8	16
	Vancomycin	MHB II	1–8	4	8
		MHB	2–8	4	8
		MHB 25	2–8	4	8
		MHB 50	2–8	4	8
		MHB 75	2–8	4	8
		BHI	2–16	8	16
	Teicoplanin	MHB II	0.5–16	4	8
		MHB	0.5–16	4	8
		MHB 25	1–16	4	16
		MHB 50	1–16	4	16
		MHB 75	1–16	4	16
		BHI	2–16	8	16
Methicillin resistant (10)	Tigecycline	MHB II	0.12–1	0.25	0.5
		MHB	0.12–0.5	0.25	0.5
		MHB 25	0.12–0.5	0.25	0.5
		MHB 50	0.25–0.5	0.25	0.5
		MHB 75	0.25–1	0.25	0.5
		BHI	0.06–0.25	0.06	0.12
	Daptomycin	MHB II	1–4	2	2
		MHB	2–4	2	4
		MHB 25	0.5–1	0.5	1
		MHB 50	0.25–0.5	0.25	0.5
		MHB 75	0.25–0.5	0.25	0.5
		BHI	32–>32	32	32
		BHI 50	2	2	2
	Vancomycin	MHB II	1–2	1	1
		MHB	1–2	1	1
		MHB 25	1–2	1	1
		MHB 50	1–2	1	2
		MHB 75	1–2	1	2
		BHI	1–4	2	2
	Teicoplanin	MHB II	0.25–8	0.25	1
		MHB	0.25–8	0.25	0.5
		MHB 25	0.25–8	0.5	1
		MHB 50	0.25–8	0.5	0.5
		MHB 75	0.25–8	0.5	1
		BHI	0.25–16	0.5	1
Methicillin susceptible (10)	Tigecycline	MHB II	0.25–0.5	0.25	0.5
		MHB	0.12–0.5	0.25	0.25
		MHB 25	0.12–0.5	0.25	0.25
		MHB 50	0.12–0.25	0.25	0.25
		MHB 75	0.25–0.5	0.25	0.5
		BHI	0.06–0.12	0.06	0.12

Continued on following page

TABLE 2—*Continued*

S. aureus phenotype (no. of isolates tested)	Antibiotic	Medium	MIC (μg/ml)		
			Range	50%	90%
	Daptomycin	MHB II	1–2	1	2
		MHB	2	2	2
		MHB 25	0.25–0.5	0.5	0.5
		MHB 50	0.12–0.5	0.25	0.5
		MHB 75	0.12–0.5	0.25	0.25
		BHI	32	32	32
		BHI 50	1–2	1	2
	Vancomycin	MHB II	0.5–1	1	1
		MHB	0.5–2	1	2
		MHB 25	0.5–2	1	2
		MHB 50	1	1	1
		MHB 75	1–2	1	1
		BHI	1–2	2	2
	Teicoplanin	MHB II	0.25–1	0.25	0.5
		MHB	0.25–1	0.25	0.5
		MHB 25	0.25–0.5	0.5	0.5
		MHB 50	0.25–0.5	0.5	0.5
		MHB 75	0.25–0.5	0.5	0.5
		BHI	0.5–1	0.5	1

decreased level of susceptibility to vancomycin and 32% of the GISA strains showed a decreased level of susceptibility to teicoplanin. In contrast, tigecycline demonstrated an increase in activity in BHI over that in MHB II (MIC_{90}s, 0.25 and 0.5 μg/ml, respectively). Daptomycin had an MIC_{90} of >32 μg/ml in unsupplemented BHI and failed to demonstrate any antibacterial activity against the GISA strains in this medium. When BHI 50 was used, daptomycin demonstrated a modest increase in activity (MIC_{90}, 16 μg/ml). Similar effects were also demonstrated by tigecycline, daptomycin, vancomycin, and teicoplanin when BHI was used to test MRSA and MSSA strains. Compared to the activities seen in MHB II, the activities of levofloxacin, erythromycin, and amoxicillin against the GISA, MRSA, and MSSA strains were slightly increased in BHI, but no trend could be established (data not shown).

The in vivo efficacies of tigecycline, daptomycin, and vancomycin determined against an MSSA, an MRSA, and a GISA strain in a murine model of bacterial infection are displayed in Table 4. Daptomycin and tigecycline exhibited similar in vivo efficacies against infections caused by the MSSA strain (strain GC 4543) (ED_{50}s, 0.12 and 0.24 mg/kg of body weight, respectively) and were approximately three to six times more efficacious than vancomycin (ED_{50}, 0.67 mg/kg). The in vivo efficacies of tigecycline and daptomycin (ED_{50}s, 0.72 and 0.87 mg/kg, respectively) were also similar against an infection with an MRSA strain (strain GC 1131). Vancomycin (ED_{50}, 2.2 mg/kg) was 2.5 and 3 times less efficacious than daptomycin and tigecycline, respectively, against the infection caused by an MRSA strain. Tigecycline was the most efficacious antibiotic tested against an infection caused by a GISA strain. Tigecycline was 3 times more efficacious than daptomycin and 16 times more active than vancomycin (ED_{50}s, 1.9, 6.1, and 31 mg/kg, respectively).

DISCUSSION

The number of strains of multidrug-resistant gram-positive bacteria has increased dramatically during the past two decades (28, 30). The emergence and spread of penicillin-resistant *S. pneumoniae*, glycopeptide-resistant enterococci, and methicillin-resistant staphylococci are now recognized as global problems (2). The isolation of *S. aureus* strains with reduced susceptibilities to glycopeptide antibiotics has been reported from Japan and other parts of Asia, the United States, and Europe (38). Although the vancomycin MICs for these isolates (≤16 μg/ml) remain below the achievable levels in serum, the clinical outcomes of these infections have been poor and additional intervention is required (8). In addition, the emergence of gram-positive strains resistant to multiple antimicrobial agents has added to the resistance problem (24). New compounds for the effective treatment of infections

TABLE 3. Effect of calcium concentration on the in vitro activities of daptomycin against quality control strains

Organism	Medium	MIC (μg/ml)
S. aureus ATCC 29213	MHB II	1
	MHB	2
	MHB 25	0.5
	MHB 50	0.25
	MHB 75	0.25
	BHI	32
	BHI 50	2
E. faecalis ATCC 29212	MHB II	16
	MHB	32
	MHB 25	4
	MHB 50	2
	MHB 75	0.5
	BHI	>32
	BHI 50	8

caused by multiresistant gram-positive species are urgently needed.

Research on antimicrobials that can be used to overcome resistance in gram-positive bacteria has produced a number of promising new compounds. Recently, quinupristin-dalfopristin has been approved for clinical use. This agent, however, has caused multiple adverse effects and has become associated with a significant emergence of resistance (19, 23). The first of a new class of antibacterials, linezolid, an oxazolidinone (4, 9, 12), has also been introduced for clinical therapy. However, the development of resistance during therapy (13, 16) and adverse effects (13, 17) have been reported. The ketolides (5, 6, 25), a glycopeptide (15, 18, 40), and new quinolones with enhanced activity against gram-positive pathogens (6) are in development.

Two promising compounds in advanced stages of clinical development are tigecycline, a glycylcycline (31, 36), and daptomycin, a semisynthetic lipopeptide (1, 37). Tigecycline has been shown to have excellent activities against gram-positive and gram-negative bacteria without any cross-resistance, including excellent activities against tetracycline-resistant organisms (14, 29). The spectrum of activity of daptomycin also includes resistant strains, but its activity is limited to gram-positive bacteria (34). Daptomycin, however, requires more free calcium than the amount present in standard MHB II to exhibit maximum in vitro activity (34). The need for higher calcium concentrations has previously been demonstrated for daptomycin as well as other calcium-dependent antibiotics (20, 21, 22). MHB II, which is recommended for use in MIC testing by the NCCLS for all antibiotics except daptomycin, does not contain sufficient calcium for daptomycin to exert its maximal antibacterial activity. No commercially available MHB which is adjusted to contain calcium at 50 mg/liter is available. Therefore, additional supplementation of MHB II with calcium is needed to comply with NCCLS recommendations for the use of media with calcium concentrations of 50 mg/liter when daptomycin is being tested. It is noteworthy that when unsupplemented MHB was tested for its calcium concentration, it was found to contain 19.3 mg/liter, which was only 0.7 mg/liter lower than the lower limit allowed in MHB II.

In this study, tigecycline demonstrated similar activities against clinical isolates of GISA, MRSA, vancomycin-resistant enterococci, and penicillin-resistant *S. pneumoniae*. Tigecycline had better activities than the comparative antibiotics against most resistant organisms when it was tested by the standard NCCLS methodology with MHB II as the test medium. The concentration of calcium (20 to 25 mg/liter) in this medium, however, is inadequate for the testing of daptomycin. Daptomycin showed increased activities when it was tested in MHB 50; the increased activities were most notable against the streptococcal and staphylococcal isolates. The activity of daptomycin approached that of tigecycline against the GISA strains only when the test medium was supplemented with excess calcium (75 mg/liter). This concentration of calcium, however, would exceed the approximate physiological levels of free calcium in human serum (45 to 55 mg/liter). It is notable that when the medium is supplemented with a previously recommended concentration of 50 mg of calcium per liter (34), daptomycin was less active than tigecycline against the GISA strains.

TABLE 4. In vivo efficacies of tigecycline, daptomycin, and vancomycin against experimental acute lethal staphylococcal infections in mice

S. aureus strain (challenge dose [no. of CFU[a]/mouse] vehicle)	Intravenous treatment	ED_{50} (mg/kg) (95% confidence limit)	MIC (μg/ml)
GC 6336, GISA (1.3×10^8, 10% mucin)	Tigecycline	1.9 (1.4–2.5)	0.25
	Daptomycin	6.1 (4.6–8.5)	4/1[b]
	Vancomycin	31 (22–45)	8
GC 1131, MRSA (1.9×10^7, 8% mucin)	Tigecycline	0.72 (0.57–0.91)	0.5
	Daptomycin	0.87 (0.69–1.1)	1/0.25[b]
	Vancomycin	2.2 (1.7–2.8)	1
GC 4543, MSSA (3.8×10^5, 5% mucin)	Tigecycline	0.24 (0.17–0.31)	0.5
	Daptomycin	0.12 (0.09–0.17)	1/0.25[b]
	Vancomycin	0.67 (0.4–2.0)	1

[a] Average number of CFU from three separate tests; variability, ≤ 0.5 $\log_{10}$.
[b] The values represent the MIC in MHB II/MIC in MHB 50.

The GISA strains are reported to express increased levels of resistance to the glycopeptide antibiotics when they are grown in BHI (3). The results of this study would concur, as the GISA strains showed increased levels of resistance to the glycopeptides antibiotics vancomycin and teicoplanin and also showed increased levels of resistance to daptomycin when they were tested in BHI. In contrast, tigecycline showed increased levels of activity against the GISA strains when it was tested in BHI. It is possible that the reduced activity of daptomycin seen in BHI could be attributed to a low calcium concentration and/or increased levels of protein binding, as protein binding has been reported to adversely affect the in vitro activity of daptomycin (33). Increasing the calcium level in BHI to 50 mg/liter resulted in only a modest increase in the activity of daptomycin, thereby indicating that protein binding was the possible cause of the decreased activity.

Tigecycline and daptomycin were more efficacious than vancomycin when they were evaluated against models of systemic murine MRSA, MSSA, and GISA infection. The differences in activity between tigecycline and daptomycin were not as pronounced in vivo as those observed in vitro in MHB II for MSSA and MRSA. This confirms that in vitro studies with daptomycin in the presence of 50 mg of calcium per liter would be a better predictor of in vivo efficacy. The two compounds demonstrated similar efficacies against infections caused by MSSA and MRSA isolates. Tigecycline, which was more active than daptomycin against GISA strains in vitro, did demonstrate a better corresponding efficacy against an infection caused by a GISA strain. The decreases in the efficacies of the three compounds, as measured by the increases in the ED_{50}s for the MSSA strains compared to those for the MRSA and the GISA strains, were much less pronounced for tigecycline than for either daptomycin or vancomycin.

Overall, when the activities of tigecycline were tested by standard NCCLS protocols in MHB II, tigecycline demonstrated good activity against drug-resistant *S. aureus* isolates and other drug-resistant gram-positive pathogens. Daptomycin also showed good activity against most of the strains tested when the calcium concentration of the medium was raised to the concentration (50 mg/liter) approved by the NCCLS. The activities of tigecycline against the GISA strains further add to

its broad spectrum of activity against drug-resistant bacteria. These results suggest that both tigecycline and daptomycin may play important roles in the treatment of infections caused by gram-positive pathogens including drug-resistant strains.

ACKNOWLEDGMENTS

We thank Heather Hartman and Eileen Lenoy for technical assistance and Eric Hayes and Christopher Sisto for determination of the calcium contents in the broth media.

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VOLUME 43, NUMBER 4 PUBLISHED MONTHLY APRIL 1999

ANTIMICROBIAL AGENTS AND CHEMOTHERAPY

AMERICAN SOCIETY FOR MICROBIOLOGY

ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Apr. 1999, p. 738–744
0066-4804/99/$04.00+0

Vol. 43, No. 4

In Vitro and In Vivo Antibacterial Activities of a Novel Glycylcycline, the 9-*t*-Butylglycylamido Derivative of Minocycline (GAR-936)

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Received 3 August 1998/Returned for modification 17 November 1998/Accepted 2 January 1999

The 9-*t*-butylglycylamido derivative of minocycline (TBG-MINO) is a recently synthesized member of a novel group of antibiotics, the glycylcyclines. This new derivative, like the first glycylcyclines, the *N,N*-dimethylglycylamido derivative of minocycline and 6-demethyl-6-deoxytetracycline, possesses activity against bacterial isolates containing the two major determinants responsible for tetracycline resistance: ribosomal protection and active efflux. The in vitro activities of TBG-MINO and the comparative agents were evaluated against strains with characterized tetracycline resistance as well as a spectrum of recent clinical aerobic and anaerobic gram-positive and gram-negative bacteria. TBG-MINO, with an MIC range of 0.25 to 0.5 μg/ml, showed good activity against strains expressing *tet*(M) (ribosomal protection), *tet*(A), *tet*(B), *tet*(C), *tet*(D), and *tet*(K) (efflux resistance determinants). TBG-MINO exhibited similar activity against methicillin-resistant *Staphylococcus aureus* (MRSA), penicillin-resistant streptococci, and vancomycin-resistant enterococci (MICs at which 90% of strains are inhibited, ≤0.5 μg/ml). TBG-MINO exhibited activity against a wide diversity of gram-negative aerobic and anaerobic bacteria, most of which were less susceptible to tetracycline and minocycline. The in vivo protective effects of TBG-MINO were examined against acute lethal infections in mice caused by *Escherichia coli*, *S. aureus*, and *Streptococcus pneumoniae* isolates. TBG-MINO, administered intravenously, demonstrated efficacy against infections caused by *S. aureus* including MRSA strains and strains containing *tet*(K) or *tet*(M) resistance determinants (median effective doses [ED_{50}s], 0.79 to 2.3 mg/kg of body weight). TBG-MINO demonstrated efficacy against infections caused by tetracycline-sensitive *E. coli* strains as well as *E. coli* strains containing either *tet*(M) or the efflux determinant *tet*(A), *tet*(B), or *tet*(C) (ED_{50}s, 1.5 to 3.5 mg/kg). Overall, TBG-MINO shows antibacterial activity against a wide spectrum of gram-positive and gram-negative aerobic and anaerobic bacteria including strains resistant to other chemotherapeutic agents. The in vivo protective effects, especially against infections caused by resistant bacteria, corresponded with the in vitro activity of TBG-MINO.

Tetracycline antibiotics were first isolated at Lederle Laboratories in 1945 and represented a significant advancement in the treatment of many infections (4, 7). However, due to an increased incidence of resistance among many bacteria (27), the use of the tetracyclines has been relegated to second- and third-line drug categories for most clinical indications (16, 25). The synthesis of new derivatives containing the *N,N*-dimethylglycylamido (DMG) substitution at the 9 position of minocycline and of 6-demethyl-6-deoxytetracycline (DMDOT) represented a significant advance in the tetracycline class of antibiotics (29). These new derivatives were named the glycylcyclines and were shown to be active against a wide spectrum of gram-positive and gram-negative bacteria, including resistant strains (5, 9, 12, 22, 31, 33, 34).

Derivatives in the minocycline series were found to be better tolerated than the DMDOT series in studies with rats (data not shown). In the present study we investigated the in vitro activity and in vivo efficacy of a new member of the glycylcyclines, TBG-MINO, the 9-*t*-butylglycylamido derivative of minocycline (Fig. 1), which was selected on the basis of its better tolerability and improved activity against tetracycline-resistant strains compared with those of DMG-DMDOT. The activity of TBG-MINO was determined against strains harboring characterized tetracycline resistance determinants and recent clinical isolates. The activities were compared with those of DMG-DMDOT, DMG-MINO, minocycline, tetracycline, and other antimicrobial agents. The efficacy of TBG-MINO was compared with those of DMG-DMDOT and minocycline against murine systemic infections caused by bacterial strains harboring characterized tetracycline resistance determinants, laboratory strains, and recent clinical isolates adapted for murine infection.

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MATERIALS AND METHODS

Organisms. Routine clinical isolates were collected from various medical centers in the United States and Canada between 1989 and 1994. Identification of each culture was done by conventional methods, as follows: gram-negative rods with the API 20E system (Analytab Products, Plainville, N.Y.) and the NF system (Remel, Lenexa, Kans.), anaerobes by the procedure outlined in the *Wadsworth Anaerobic Bacteriology Manual* (30), enterococci by biochemical tests as recommended by Facklam and Collins (6), streptococci with the API 20 Strep system (Analytab Products), and staphylococci with the Staph Trac system (Analytab Products). *Staphylococcus aureus* was also confirmed by a coagulase-test. Methicillin-resistance in *S. aureus* was determined with a plate containing oxacillin at 6 μg/ml, as described in the *Manual of Clinical Microbiology* (28). Penicillin-resistant (MICs, ≥2 μg/ml) *Streptococcus pneumoniae* isolates were obtained from A. Barry, Clinical Microbiology Institute, Tualatin, Oreg., and S. Block, Bardstown, Ky. Strains with tetracycline resistance determinants and the vancomycin-resistant enterococci were obtained from the sources described previously (31). All isolates were stored frozen in skim milk at −70°C.

Antibiotics. Standard powders of TBG-MINO, DMG-MINO, DMG-DMDOT, vancomycin, minocycline, and tetracycline were obtained from Wyeth-Ayerst Laboratories, Pearl River, N.Y.; erythromycin was obtained from Sigma Chemical Co., St. Louis, Mo.; ciprofloxacin was obtained from Bayer Laboratories, West Haven, Conn.; ceftazidime was obtained from Glaxo Group Research, Ware, Herts, United Kingdom; and imipenem was obtained from Merck & Co., West Point, Pa.

In vitro susceptibility testing. The activities of the antibiotics were determined by the agar dilution method by following the recommendations of the National Committee for Clinical Laboratory Standards (20, 21). Mueller-Hinton II agar was used to test nonfastidious aerobic bacteria. The medium was supplemented with 5% sheep blood for the testing of streptococcal isolates and 15 μg of β-NAD per ml, 15 μg of hematin per ml, and 5 mg of yeast extract per ml for the testing

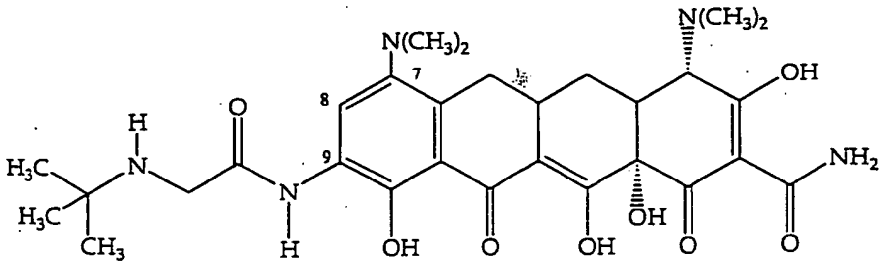

FIG. 1. Chemical structure of TBG-MINO.

of *Haemophilis influenzae* and *Moraxella catarrhalis*. GC agar supplemented with 1% hemoglobin and 1% IsoVitaleX was used to test *Neisseria gonorrhoeae*. Anaerobic bacteria were tested on Wilkins Chalgren agar supplemented with 5% lysed sheep blood and 0.001% vitamin K. The inocula, which were adjusted to the recommended densities (10^7 CFU/ml for aerobes and 10^8 CFU/ml for anaerobes), were applied to the surfaces of the agar plates with a Steers replicator. Test plates were incubated at 35°C for 18 to 24 h in ambient air for nonfastidious aerobic bacteria and streptococci and in CO_2 for *N. gonorrhoeae*, *H. influenzae*, and *M. catarrhalis*. Anaerobic bacteria were incubated in an anaerobic chamber (Coy Laboratories, Ann Arbor, Mich.) at 35°C for 48 h. The MIC was defined as the lowest concentration of the antimicrobial agent that completely inhibited the growth of the organism as detected by the unaided eye.

In vivo efficacy against murine infections. The therapeutic effects of the antibiotics were determined against acute lethal infections in mice (3) caused by minocycline-susceptible and minocycline-resistant gram-positive and gram-negative bacteria. Female CD-1 mice from Charles River Laboratories (weight, 20 ± 2 g each) were challenged by intraperitoneal injection of 0.5 ml of a bacterial suspension in either 5% hog gastric mucin or broth (10 to 100 50% lethal doses). Five to six doses of the antibiotic in phosphate-buffered saline (0.01 M; pH 7.4) were administered intravenously (0.2 ml) or orally (0.5 ml) at 0.5 h postinfection. For mice infected with *Escherichia coli* JC3272 Tc^r *tet*(B), a second dose of the antibiotic was given 3 h later. In each test, five animals were treated with each dose. All the untreated controls died within 48 h of infection. The median effective dose (ED_{50}) was determined by probit analysis of the 7-day survival ratios pooled from three separate tests (8).

RESULTS

In vitro activity against tetracycline-resistant strains. The in vitro activity of TBG-MINO against prototype strains possessing characterized tetracycline resistance mechanisms is summarized in Table 1. TBG-MINO had similar activity (MICs, ≤0.5 μg/ml) against tetracycline-susceptible and tetracycline-resistant *E. coli* strains carrying the efflux resistance determinants *tet*(A), *tet*(B), *tet*(C), and *tet*(D) and the strain carrying the ribosomal protection resistance determinant *tet*(M). TBG-MINO had activity similar to those of DMG-MINO and DMG-DMDOT against *E. coli* strains containing the *tet*(B) and *tet*(D) efflux resistance determinant and the ribosomal protection resistance determinant *tet*(M); however, TBG-MINO was more active than DMG-MINO and DMG-DMDOT against *E. coli* strains containing efflux resistance determinants *tet*(A) and *tet*(C). Minocycline demonstrated poorer activity (MIC range, 4 to >32 μg/ml) against all of the *E. coli* strains carrying the resistance determinants. TBG-MINO, with MICs of ≤0.5 μg/ml, was as active as DMG derivatives against the *tet*(K) (efflux)- and *tet*(M)-containing *S. aureus* strains. Minocycline was slightly more active than the glycylcyclines against *tet*(K)-containing *S. aureus* but had poorer activity against the three *S. aureus* strains containing *tet*(M).

In vitro activity against recent clinical isolates. TBG-MINO showed good activity against isolates of methicillin-resistant *S. aureus* (MRSA) and methicillin-resistant coagulase-negative staphylococci (MICs at which 90% of isolates are inhibited [MIC_{90}s], ≤1 μg/ml). This activity was similar to that of minocycline and was 2 to 3 dilutions lower than those of DMG-MINO and DMG-DMDOT (Table 2). Against methicillin-

TABLE 1. In vitro activities of TBG-MINO, DMG-MINO, DMG-DMDOT, minocycline, and tetracycline against strains with characterized tetracycline resistance determinants

Organism	Strain	Resistance determinant	MIC (μg/ml)				
			TBG-MINO	DMG-MINO	DMG-DMDOT	Minocycline	Tetracycline
E. coli	UBMS 88-1	*tet*(B)	0.5	0.5	0.5	16	>32
E. coli	MC4100	*tet*(B)	0.5	0.5	0.5	8	>32
E. coli	J3272, pRP1	*tet*(A)	0.5	2	2	4	32
E. coli	J3272, pBR322	*tet*(C)	0.25	2	2	4	>32
E. coli	J3272, pRA1	*tet*(D)	0.25	0.25	0.25	8	>32
E. coli	UBMS 90-4	*tet*(M)	0.25	0.25	0.25	>32	>32
E. coli	UBMS 90-5	Sensitive	0.25	0.5	0.25	1	1
E. coli	ATCC 25922	Control	0.25	0.25	0.25	0.5	1
S. aureus	UBMS 88-7	*tet*(K)	0.5	1	1	0.25	>32
S. aureus	UBMS 88-5	*tet*(M)	0.5	0.25	0.25	4	>32
S. aureus	UBMS 90-1	*tet*(M)	0.25	0.25	0.12	4	>32
S. aureus	UBMS 90-2	*tet*(M)	0.25	0.25	0.25	2	32
S. aureus	UBMS 90-3	Sensitive	0.25	0.25	0.12	0.06	0.12
S. aureus	ATCC 29213	Control	0.5	0.25	0.25	0.06	0.25
S. aureus	Smith	Sensitive	0.25	0.25	0.12	0.06	0.12
E. faecalis	UBMS 90-6	*tet*(M)	0.25	0.12	0.25	16	>32
E. faecalis	ATCC 29212	Control	0.25	0.12	0.12	1	8
N. gonorrhoeae	6418	*tet*(M)	1	1	1	16	>32

TABLE 2. In vitro activities of TBG-MINO and comparative antibiotics against gram-positive isolates

Organism (no. of isolates)	Antibiotic	MIC (μg/ml)		
		Range	50%	90%
Staphylococcus aureus, methicillin resistant (12)	TBG-MINO	0.25–1	0.5	0.5
	DMG-MINO	0.12–2	0.25	2
	DMG-DMDOT	0.25–2	0.5	2
	Minocycline	0.03–4	0.12	1
	Tetracycline	0.25–>32	0.5	>32
	Ciprofloxacin	0.25–>32	8	32
	Vancomycin	0.5–2	1	1
	Erythromycin	4–>32	>32	>32
Staphylococcus aureus, methicillin susceptible (13)	TBG-MINO	0.5	0.5	0.5
	DMG-MINO	0.25–0.5	0.5	0.5
	DMG-DMDOT	0.5	0.5	0.5
	Minocycline	0.12	0.12	0.12
	Tetracycline	0.5	0.5	0.5
	Ciprofloxacin	0.5–1	0.5	1
	Vancomycin	0.5–1	1	1
	Erythromycin	0.5–1	0.5	0.5
Coagulase-negative staphylococci, methicillin resistant (13)	TBG-MINO	0.25–2	0.5	1
	DMG-MINO	0.25–8	0.5	4
	DMG-DMDOT	0.12–8	1	8
	Minocycline	0.12–1	0.5	1
	Tetracycline	0.25–>32	4	>32
	Ciprofloxacin	0.25–>32	0.5	32
	Vancomycin	1–2	2	2
	Erythromycin	0.12–>32	>32	>32
Coagulase-negative staphylococci, methicillin susceptible (16)	TBG-MINO	0.12–0.5	0.25	0.5
	DMG-MINO	0.12–0.5	0.25	0.5
	DMG-DMDOT	0.12–1	0.25	0.5
	Minocycline	0.06–0.5	0.12	0.25
	Tetracycline	0.12–32	0.5	32
	Ciprofloxacin	0.12–1	0.25	0.5
	Vancomycin	1–2	1	2
	Erythromycin	0.12–>32	0.12	1
Enterococcus faecalis (11)	TBG-MINO	0.12–0.5	0.25	0.5
	DMG-MINO	0.06–0.25	0.25	0.25
	DMG-DMDOT	0.12–0.5	0.25	0.25
	Minocycline	0.06–16	8	8
	Tetracycline	0.25–32	32	32
	Ciprofloxacin	1–32	1	2
	Vancomycin	1–4	2	2
	Erythromycin	1–16	1	8
Enterococcus faecium (11)	TBG-MINO	0.12–0.25	0.25	0.25
	DMG-MINO	0.06–0.12	0.12	0.12
	DMG-DMDOT	0.12–0.25	0.12	0.12
	Minocycline	0.06–16	0.06	4
	Tetracycline	0.25–>32	0.25	32
	Ciprofloxacin	1–4	4	4
	Vancomycin	0.25–2	1	2
	Erythromycin	0.5–>32	4	>32
Enterococcus spp., vancomycin resistant (10)	TBG-MINO	0.12–0.25	0.25	0.25
	DMG-MINO	0.06–0.25	0.12	0.25
	DMG-DMDOT	0.12–0.25	0.12	0.25
	Minocycline	0.06–8	0.06	8
	Tetracycline	0.25–>32	0.25	>32
	Ciprofloxacin	0.5–4	4	4
	Vancomycin	>32	>32	>32
	Erythromycin	>32	>32	>32
Streptococcus pneumoniae, penicillin resistant (10)	TBG-MINO	0.06–0.25	0.12	0.12
	DMG-MINO	0.03–0.12	0.06	0.06
	DMG-DMDOT	0.06–0.12	0.12	0.12
	Minocycline	0.12–8	4	4
	Tetracycline	0.5–32	32	32
	Vancomycin	0.25–0.5	0.5	0.5
Streptococcus pneumoniae, penicillin susceptible (10)	TBG-MINO	0.06–0.12	0.06	0.12
	DMG-MINO	0.06–0.25	0.06	0.12
	DMG-DMDOT	0.06–0.5	0.06	0.12
	Minocycline	0.06–0.5	0.12	0.12
	Tetracycline	0.12–4	0.25	0.5
	Vancomycin	0.12–1	0.5	0.5
Streptococcus pyogenes (10)	TBG-MINO	0.12–0.5	0.12	0.25
	DMG-MINO	0.06–0.12	0.12	0.12
	DMG-DMDOT	0.12	0.12	0.12
	Minocycline	0.06–0.25	0.06	0.12
	Tetracycline	0.25–16	0.25	0.25
	Vancomycin	0.5	0.5	0.5
Streptococcus agalactiae (10)	TBG-MINO	0.12–0.5	0.12	0.25
	DMG-MINO	0.12–0.5	0.12	0.5
	DMG-DMDOT	0.12–1	0.12	0.25
	Minocycline	0.12–16	16	16
	Tetracycline	0.25–32	32	32
	Vancomycin	0.5–1	0.5	0.5

usceptible staphylococci, the three glycylcycline derivatives had quivalent activities (MICs, ≤0.5 μg/ml). TBG-MINO and the DMG derivatives demonstrated activity against *Enterococcus aecalis* and *Enterococcus faecium*, including vancomycin-resistant strains (MIC_{90}s, ≤0.5 μg/ml). The three glycylcyclines, minocycline, and tetracycline exhibited good activity against *Streptococcus pyogenes* and penicillin-susceptible *S. pneumoniae*; however, TBG-MINO and the DMG derivatives were 32 to 64 times more active than minocycline against *Streptococcus agalactiae* and penicillin-resistant *S. pneumoniae*. No differences in the activity of TBG-MINO between penicillin-susceptible and penicillin-resistant *S. pneumoniae* isolates were noted. In general, TBG-MINO, with MICs of ≤1 μg/ml, displayed greater activity than the other comparative antibiotics, vancomycin, ciprofloxacin, and erythromycin, against most of the staphylococcal and enterococcal isolates tested.

TBG-MINO, with a range of MICs of 0.5 to 8 μg/ml, was 4 to 32 times more active than minocycline against clinical isolates of *E. coli*, *Shigella* spp., *Citrobacter diversus*, *Salmonella* spp., *Providencia* spp., *Morganella morganii*, and *N. gonorrhoeae* (Table 3). TBG-MINO was generally as active or more active than minocycline against most strains of *Klebsiella* spp., *Citrobacter freundii*, *Enterobacter* spp., *Serratia marcescens*, *Proteus urabilis*, *Proteus vulgaris*, *Burkholderia cepacia*, and *Pseudomonas aeruginosa*. In general, the three glycylcyclines demonstrated similar activities against gram-negative isolates; however, greater activity was observed with TBG-MINO than with DMG-MINO or DMG-DMDOT (MIC_{90}s, ≤0.5 versus 4 μg/ml, respectively) against *E. coli* strains for which minocycline MICs were elevated (MIC_{90}, 16 μg/ml). TBG-MINO, DMG-MINO, and DMG-DMDOT were generally less active than ciprofloxacin, imipenem, and ceftazidime against most gram-negative bacteria. However, organisms resistant to these antibiotics showed no cross-resistance with the glycylcyclines.

TBG-MINO and the other glycylcycline derivatives, with a range of MICs of 0.12 to 2 μg/ml, were more active than minocycline against *Bacteroides* spp., *Prevotella* spp., *Clostridium difficile*, and anaerobic gram-positive cocci (Table 4). For some members of the *Bacteroides fragilis* group, the MICs of TBG-MINO but not those of DMG-MINO or DMG-DMDOT were found to be elevated (1 to 2 μg/ml). In general, the three glycylcyclines were more active than cefoxitin but were less active than imipenem against most of the anaerobic bacteria tested.

In vivo efficacy. Administered as a single intravenous dose, TBG-MINO showed efficacy against infections caused by tetracycline-susceptible and tetracycline-resistant *S. aureus* and *E. coli* strains in mice (Table 5 and 6). Against an infection with

TABLE 3. In vitro activities of TBG-MINO and comparative antibiotics against gram-negative isolates

Organism (no. of isolates)	Antibiotic	MIC (μg/ml)		
		Range	50%	90%
Escherichia coli (minocycline MIC, ≥1 μg/ml (32)	TBG-MINO	0.25–1	0.5	0.5
	DMG-MINO	0.25–4	0.5	4
	DMG-DMDOT	0.25–4	1	4
	Minocycline	1–32	8	16
	Tetracycline	2–>32	>32	>32
	Ciprofloxacin	0.008–32	0.008	0.015
	Imipenem	0.06–0.25	0.12	0.12
	Ceftazidime	0.06–1	0.12	0.25
Escherichia coli (minocycline MIC, ≤0.5 μg/ml) (14)	TBG-MINO	0.25–0.5	0.5	0.5
	DMG-MINO	0.25–0.5	0.25	0.5
	DMG-DMDOT	0.5	0.5	0.5
	Minocycline	0.25–0.5	0.5	0.5
	Tetracycline	1–2	1	2
	Ciprofloxacin	≤0.004–0.25	≤0.004	0.03
	Imipenem	0.06–0.12	0.12	0.12
	Ceftazidime	0.06–0.25	0.12	0.12
Shigella spp. (26)	TBG-MINO	0.12–0.5	0.25	0.5
	DMG-MINO	0.12–1	0.25	0.5
	DMG-DMDOT	0.12–1	0.5	0.5
	Minocycline	0.25–16	2	4
	Tetracycline	1–>32	>32	>32
	Ciprofloxacin	≤0.004–0.015	≤0.004	0.008
	Imipenem	0.06–0.5	0.12	0.25
	Ceftazidime	0.06–0.12	0.12	0.12
Klebsiella pneumoniae (10)	TBG-MINO	0.5–2	1	2
	DMG-MINO	0.5–1	1	1
	DMG-DMDOT	0.5–1	1	1
	Minocycline	1–4	2	4
	Tetracycline	1–4	2	2
	Ciprofloxacin	0.008–0.03	0.03	0.03
	Imipenem	0.12–0.5	0.12	0.25
	Ceftazidime	0.06–0.5	0.12	0.12
Klebsiella oxytoca (10)	TBG-MINO	1	1	1
	DMG-MINO	1	1	1
	DMG-DMDOT	1	1	1
	Minocycline	2–8	2	2
	Tetracycline	2–>32	2	2
	Ciprofloxacin	0.008–0.03	0.015	0.015
	Imipenem	0.12–0.25	0.12	0.25
	Ceftazidime	0.06–0.5	0.12	0.12
Citrobacter freundii (10)	TBG-MINO	0.5–8	1	2
	DMG-MINO	0.5–8	1	2
	DMG-DMDOT	1–8	1	1
	Minocycline	1–32	4	4
	Tetracycline	1–16	2	2
	Ciprofloxacin	≤0.004–16	0.015	0.12
	Imipenem	0.25–2	0.5	1
	Ceftazidime	0.12–>32	0.5	8
Citrobacter diversus (10)	TBG-MINO	0.5–2	1	1
	DMG-MINO	0.5–2	1	1
	DMG-DMDOT	1–2	1	1
	Minocycline	1–4	2	4
	Tetracycline	2–8	2	4
	Ciprofloxacin	≤0.004–0.06	0.008	0.06
	Imipenem	0.06–12	0.06	0.12
	Ceftazidime	0.12–0.5	0.12	0.5
Salmonella spp. (14)	TBG-MINO	0.25–2	1	1
	DMG-MINO	0.5–4	0.5	1
	DMG-DMDOT	0.5–4	0.5	1
	Minocycline	0.5–32	2	16
	Tetracycline	1–>32	2	>32
	Ciprofloxacin	≤0.004–0.03	0.015	0.03
	Imipenem	0.06–0.25	0.12	0.25
	Ceftazidime	0.12–0.5	0.25	0.5
Serratia marcescens (10)	TBG-MINO	4–8	4	4
	DMG-MINO	4–8	4	8
	DMG-DMDOT	4–8	4	4
	Minocycline	4–8	8	8
	Tetracycline	8–>32	32	>32
	Ciprofloxacin	0.008–2	0.12	0.25
	Imipenem	0.25–2	0.5	2
	Ceftazidime	0.12–1	0.25	0.5
Enterobacter cloacae (10)	TBG-MINO	1–2	1	2
	DMG-MINO	1–2	1	2
	DMG-DMDOT	1–2	1	2
	Minocycline	2–4	4	4
	Tetracycline	2–4	4	4
	Ciprofloxacin	≤0.004–0.06	0.03	0.03
	Imipenem	0.25	0.25	0.25
	Ceftazidime	0.12–>32	0.25	>32
Enterobacter aerogenes (10)	TBG-MINO	1	1	1
	DMG-MINO	1	1	1
	DMG-DMDOT	1	1	1
	Minocycline	2	2	2
	Tetracycline	2	2	2
	Ciprofloxacin	≤0.004–0.03	0.015	0.03
	Imipenem	0.25–2	0.25	2
	Ceftazidime	0.12–>32	0.12	>32
Providencia spp. (10)	TBG-MINO	4–8	4	8
	DMG-MINO	2–8	8	8
	DMG-DMDOT	4–8	4	8
	Minocycline	4–>32	16	>32
	Tetracycline	4–>32	>32	>32
	Ciprofloxacin	≤0.004–0.25	0.03	0.25
	Imipenem	0.25–2	1	2
	Ceftazidime	0.03–4	0.06	4
Proteus mirabilis (15)	TBG-MINO	2–8	4	8
	DMG-MINO	1–16	4	8
	DMG-DMDOT	0.12–2	1	1
	Minocycline	2–32	8	16
	Tetracycline	1–32	16	32
	Ciprofloxacin	0.008–0.06	0.06	0.06
	Imipenem	0.003–0.12	0.06	0.12
	Ceftazidime	0.015–0.06	0.03	0.03
Proteus vulgaris (15)	TBG-MINO	1–4	4	4
	DMG-MINO	0.5–4	1	2
	DMG-DMDOT	0.25–1	0.5	1
	Minocycline	0.5–8	2	4
	Tetracycline	0.5–>32	8	32
	Ciprofloxacin	0.008–0.25	0.015	0.12
	Imipenem	0.03–0.12	0.06	0.12
	Ceftazidime	0.015–0.25	0.03	0.06
Morganella morganii (10)	TBG-MINO	2–8	4	4
	DMG-MINO	1–4	4	4
	DMG-DMDOT	1–4	2	2
	Minocycline	2–>32	4	16
	Tetracycline	2–>32	2	>32
	Ciprofloxacin	≤0.004–1	0.008	0.03
	Imipenem	2–4	2	4
	Ceftazidime	0.06–32	0.12	32
Pseudomonas aeruginosa (10)	TBG-MINO	8–16	16	16
	DMG-MINO	4–8	8	8
	DMG-DMDOT	4–16	8	8
	Minocycline	2–8	8	8
	Tetracycline	8–>32	16	>32
	Ciprofloxacin	0.12–2	0.25	2
	Imipenem	0.5–2	1	1
	Ceftazidime	0.5–32	2	16
Burkholderia cepacia (10)	TBG-MINO	0.5–4	2	4
	DMG-MINO	0.5–4	1	4
	DMG-DMDOT	0.5–4	2	4
	Minocycline	0.06–2	0.5	2
	Tetracycline	1–>32	2	4
	Ciprofloxacin	0.03–4	0.12	2
	Imipenem	0.06–8	4	8
	Ceftazidime	0.5–4	2	4
Stenotrophomonas maltophilia (10)	TBG-MINO	1–4	2	4
	DMG-MINO	0.5–4	1	2
	DMG-DMDOT	2–8	4	8
	Minocycline	0.06–0.5	0.12	0.25
	Tetracycline	8–16	16	16
	Ciprofloxacin	1–4	2	4
	Imipenem	>32	>32	>32
	Ceftazidime	4–>32	8	>32

Continued on following page

TABLE 3—*Continued*

Organism (no. of isolates)	Antibiotic	MIC (μg/ml)		
		Range	50%	90%
Moraxella catarrhalis (14)	TBG-MINO	0.12–0.25	0.12	0.25
	DMG-MINO	0.06–0.12	0.12	0.12
	DMG-DMDOT	0.12–0.25	0.12	0.25
	Minocycline	0.008–0.06	0.03	0.06
	Tetracycline	0.06–0.25	0.12	0.25
	Ciprofloxacin	0.03–0.06	0.03	0.06
	Imipenem	0.008–0.06	0.015	0.06
	Ceftazidime	0.015–0.12	0.015	0.06
Neisseria gonorrhoeae (22)	TBG-MINO	0.25–1	0.5	1
	DMG-MINO	0.12–1	0.25	0.5
	DMG-DMDOT	0.25–1	0.5	1
	Minocycline	0.25–>32	0.5	32
	Tetracycline	0.5–>32	1	>32
	Ciprofloxacin	≤0.004	≤0.004	≤0.004
	Imipenem	0.015–0.12	0.06	0.12
	Ceftazidime	0.015–0.25	0.03	0.06
Haemophilus influenzae (15)	TBG-MINO	0.25–1	0.5	1
	DMG-MINO	0.25–0.5	0.25	0.5
	DMG-DMDOT	0.25–0.5	0.5	0.5
	Minocycline	0.12–0.25	0.12	0.25
	Tetracycline	0.12–8	0.25	0.5
	Ciprofloxacin	≤0.004–0.03	0.015	0.03
	Imipenem	1–8	2	4
	Ceftazidime	0.015–0.25	0.12	0.12

S. aureus Smith, a tetracycline-susceptible strain, all three compounds, TBG-MINO, DMG-DMDOT, and minocycline, displayed efficacy (ED_{50}s, 0.64, 0.51, and 0.53 mg/kg of body weight, respectively) when they were administered intravenously; however, when they were administered orally, TBG-MINO and DMG-DMDOT were 40- to 60-fold less efficacious (Table 5). In contrast, when administered orally minocycline exhibited efficacy equivalent to that achieved when it was administered intravenously against *S. aureus* Smith infection (ED_{50}, 0.52 mg/kg). Due to the poor efficacy in mice noted when the drugs were given by the oral route, other in vivo tests were performed with only intravenous administration. TBG-MINO and DMG-DMDOT were moderately more efficacious than minocycline against an infection with *S. aureus* UBMS 90-2 [a *tet*(M) (ribosomal protection)-containing strain] (Table 5). TBG-MINO, DMG-DMDOT, and minocycline had comparable efficacies against an infection caused by *S. aureus* UBMS 88-7, a *tet*(K) efflux resistance determinant-containing strain (ED_{50}s, 2.1, 3.1, and 2.0 mg/kg, respectively). TBG-MINO and DMG-DMDOT showed protective efficacy against an infection caused by *S. aureus* NEMC 89-4 (a tetracycline-susceptible, methicillin-resistant strain), but minocycline was slightly more effective. Against infections caused by an MRSA strain containing the *tet*(M) resistance determinant (strain ID 4729) and an MRSA strain carrying both *tet*(M) and *tet*(K) resistance determinants (strain ID 2371), TBG-MINO and DMG-DMDOT showed efficacies which exceeded that of minocycline by approximately two and five times, respectively. Comparable efficacies against infections caused by *S. pneumoniae* were obtained with TBG-MINO and DMG-DMDOT, regardless of the strain's susceptibility to penicillin (range of ED_{50}s, 0.53 to 1.9 mg/kg). Minocycline was slightly less effective against infections caused by penicillin-susceptible *S. pneumoniae* and was >30 times less effective than the glycylcyclines against a penicillin-resistant *S. pneumoniae* infection (ED_{50}, 20 mg/kg).

TBG-MINO, DMG-DMDOT, and minocycline were observed to have similar efficacies against an infection caused by the tetracycline-susceptible strain *E. coli* 311, with ED_{50}s of 1.7, 1.5, and 3.2 mg/kg, respectively. Against infections caused by *E. coli* strains containing *tet*(A) or *tet*(C) efflux resistance determinants, TBG-MINO (ED_{50}s, 1.6 and 1.5 mg/kg, respectively) exhibited efficacy that was approximately three times that of DMG-DMDOT and more than nine times that of minocycline. Against an infection caused by *E. coli* UBMS 90-4, a laboratory strain in which the *tet*(M) resistance determinant mechanism was inserted, both TBG-MINO and DMG-DMDOT, with ED_{50}s of 3.5 and 2.1 mg/kg, respectively, demonstrated good efficacy, while minocycline was not therapeutically effective at doses of up to 32 mg/kg. Intravenous administration of TBG-MINO or DMG-DMDOT resulted in good

TABLE 4. In vitro activities of TBG-MINO and comparative antibiotics against anaerobic bacteria

Organism (no. of isolates)	Antibiotic	MIC (μg/ml)		
		Range	50%	90%
Bacteroides fragilis group (12)	TBG-MINO	0.25–2	0.5	2
	DMG-MINO	0.25–0.5	0.25	0.5
	DMG-DMDOT	0.25–0.5	0.25	0.5
	Minocycline	0.06–4	2	4
	Cefoxitin	2–>32	16	>32
	Imipenem	≤0.06–2	0.12	2
Bacteroides fragilis (14)	TBG-MINO	0.5–8	2	2
	DMG-MINO	0.25–2	1	1
	DMG-DMDOT	0.5–2	1	2
	Minocycline	≤0.06–8	8	8
	Cefoxitin	1–8	8	8
	Imipenem	≤0.06–0.25	≤0.06	0.12
Prevotella spp. (11)	TBG-MINO	0.12–1	0.5	1
	DMG-MINO	≤0.06–0.5	0.25	0.5
	DMG-DMDOT	≤0.06–2	0.5	2
	Minocycline	≤0.06–16	8	16
	Cefoxitin	0.25–4	1	2
	Imipenem	≤0.06	≤0.06	≤0.06
Clostridium difficile (10)	TBG-MINO	≤0.06–0.25	0.12	0.12
	DMG-MINO	≤0.06–0.12	0.12	0.12
	DMG-DMDOT	≤0.06–0.12	0.12	0.12
	Minocycline	≤0.06–4	0.03	4
	Cefoxitin	>32	>32	>32
	Imipenem	2–16	4	4
Clostridium perfringens (10)	TBG-MINO	0.12–4	0.5	1
	DMG-MINO	0.12–4	0.25	2
	DMG-DMDOT	0.12–4	0.25	2
	Minocycline	≤0.06–8	≤0.06	4
	Cefoxitin	0.25–1	0.5	1
	Imipenem	≤0.06	≤0.06	≤0.06
Anaerobic gram-positive cocci (15)	TBG-MINO	≤0.06–0.25	0.12	0.25
	DMG-MINO	≤0.06–0.12	≤0.06	0.12
	DMG-DMDOT	≤0.06–0.5	0.12	0.5
	Minocycline	0.12–16	4	16
	Cefoxitin	≤0.06–16	0.12	16
	Imipenem	≤0.06–1	≤0.06	1

TABLE 5. In vivo activities of TBG-MINO, DMG-DMDOT, and minocycline against experimental acute lethal *S. aureus* Smith infection in mice[a]

Antibiotic	Route	ED_{50} (mg/kg) (95% confidence limit)	MIC (μg/ml)
TBG-MINO	Intravenous	0.64 (0.51–0.80)	0.25
TBG-MINO	Oral	36 (28–45)	0.25
DMG-DMDOT	Intravenous	0.51 (0.41–0.64)	0.12
DMG-DMDOT	Oral	21 (16–26)	0.12
Minocycline	Intravenous	0.53 (0.40–0.70)	0.06
Minocycline	Oral	0.52 (0.40–0.69)	0.06

[a] Challenge dose, 6.2×10^5 CFU/mouse.

TABLE 6. In vivo activities of TBG-MINO, DMG-DMDOT, and minocycline against experimental acute lethal infections in mice

Infection (resistance determinant or resistance; challenge dose [CFU/mouse])	Intravenous treatment	ED_{50} (mg/kg) (95% confidence limit)	MIC (μg/ml)
Staphylococcus aureus UBMS 90-2 (*tet*(M), ribosomal protection; 7.9×10^7)	TBG-MINO	1.0 (0.87–1.3)	0.12
	DMG-DMDOT	0.68 (0.56–0.81)	0.12
	Minocycline	1.8 (1.5–2.2)	2.0
Staphylococcus aureus UBMS 88-7,649(pUB111) (*tet*(K), efflux; 9.0×10^7)	TBG-MINO	2.1 (1.8–2.6)	0.5
	DMG-DMDOT	3.1 (2.5–3.7)	1.0
	Minocycline	2.0 (1.6–2.4)	0.25
Staphylococcus aureus NEMC 89-4 (MRSA; 5.3×10^7)	TBG-MINO	0.79 (0.64–0.97)	0.50
	DMG-DMDOT	0.48 (0.39–0.59)	0.25
	Minocycline	0.31 (0.25–0.38)	0.12
Staphylococcus aureus ID 4729 (MRSA, *tet*(M); 1.3×10^8)	TBG-MINO	0.84 (0.69–1.0)	0.5
	DMG-DMDOT	0.53 (0.43–0.64)	0.25
	Minocycline	1.6 (1.3–2.0)	4.0
Staphylococcus aureus ID 2371 (MRSA, *tet*(M), *tet*(K); 1.3×10^8)	TBG-MINO	2.3 (1.9–2.7)	1.0
	DMG-DMDOT	3.0 (2.4–3.6)	2.0
	Minocycline	16 (13–20)	4.0
Streptococcus pneumoniae ATCC 6301 (penicillin susceptible; 3.3×10^1)	TBG-MINO	1.3 (1.1–1.6)	0.12
	DMG-DMDOT	1.3 (1.1–1.6)	0.06
	Minocycline	3.9 (3.2–4.8)	0.12
Streptococcus pneumoniae ATCC 10015 (penicillin susceptible; 1.5×10^1)	TBG-MINO	1.7 (1.4–2.2)	0.12
	DMG-DMDOT	1.9 (1.5–2.4)	0.12
	Minocycline	3.5 (2.8–4.4)	0.12
Streptococcus pneumoniae GS 1894 (penicillin resistant; 3.7×10^1)	TBG-MINO	0.61 (0.48–0.77)	0.12
	DMG-DMDOT	0.53 (0.42–0.67)	0.12
	Minocycline	20 (16–26)	4
Escherichia coli 311 (susceptible; 2.3×10^6)	TBG-MINO	1.7 (1.4–2.1)	0.5
	DMG-DMDOT	1.5 (1.2–1.8)	0.5
	Minocycline	3.2 (2.6–4.0)	1.0
Escherichia coli J3272 (pRP1) (*tet*(A), efflux; 1.6×10^7)	TBG-MINO	1.6 (1.0–2.6)	0.5
	DMG-DMDOT	4.6 (2.9–7.5)	2.0
	Minocycline	16.0 (9.8–26.0)	4.0
Escherichia coli J3272(pBR322) (*tet*(C), efflux; 2.6×10^7)	TBG-MINO	1.5 (1.3–1.9)	0.25
	DMG-DMDOT	5.0 (4.1–6.4)	2.0
	Minocycline	14.0 (11.0–17.0)	4.0
Escherichia coli UBMS 90-4 (*tet*(M), ribosomal protection; 6.6×10^7)	TBG-MINO	3.5 (2.8–4.3)	0.25
	DMG-DMDOT	2.1 (1.8–2.6)	0.25
	Minocycline	>32.0	>32.0
Escherichia coli UBMS 88-1, J3272TcR (*tet*(B), efflux; 3.9×10^7)	TBG-MINO	3.9 (3.2–4.9)	0.5
	DMG-DMDOT	3.1 (2.5–3.8)	0.5
	Minocycline	>32.0	32.0
Escherichia coli NEMC 87-30 (minocycline resistant; 5.3×10^7)	TBG-MINO	1.6 (1.3–1.9)	0.5
	DMG-DMDOT	2.0 (1.7–2.4)	0.5
	Minocycline	>32.0	32.0

efficacy against an infection caused by *E. coli* UBMS 88-1, a strain carrying the *tet*(B) efflux resistance determinant, while minocycline was not efficacious. Both TBG-MINO and DMG-DMDOT showed efficacy (ED_{50}s, ≤2.0 mg/kg) against an infection caused by a minocycline-resistant *E. coli* clinical isolate (NEMC 87-30).

DISCUSSION

Previous studies (5, 9, 12, 22, 31, 33, 34) demonstrated that the DMG modification of the 9 position of the tetracycline molecule (29), i.e., DMG-MINO and DMG DMDOT, resulted in drugs that have the ability to overcome the two major mechanisms responsible for tetracycline resistance, i.e., ribosomal protection or active efflux of drug out of the bacterial cell (1, 2, 13–15, 24, 26, 27). TBG-MINO, the 9-*t*-butylglycylamido derivative of minocycline, a recently synthesized member of the glycylcycline family of compounds, possesses a spectrum of activity similar to those DMG-MINO and DMG-DMDOT against most of the strains carrying the tetracycline resistance determinants. However, TBG-MINO has improved in vitro and in vivo activities against *E. coli* strains carrying the *tet*(A) or *tet*(C) resistance determinant.

The activity of TBG-MINO matched the activities of DMG-MINO and DMG-DMDOT against recent clinical gram-negative and -positive aerobic and anaerobic isolates, including minocycline- and tetracycline-resistant isolates. Differences in activities between TBG-MINO, DMG-MINO, and DMG-DMDOT were noted against some strains of *E. coli*, against which TBG-MINO was more active than DMG-MINO or DMG-DMDOT. Because TBG-MINO demonstrated better activity

vhen it was tested against prototype strains of *E. coli* with *et*(A) or *tet*(C) resistance determinants, it is possible that some of these clinical isolates may contain one or both of these resistance determinants. The MIC_{90}s of TBG-MINO for MRSA and methicillin-resistant coagulase-negative staphylococci were also lower. The MICs of DMG-DMDOT and DMG-MINO were elevated for two of the clinical MRSA strains, which contained both *tet*(K) and *tet*(M) resistance determinants, but these strains were more sensitive to TBG-MINO (data not shown). Because all three glycylcyclines showed good activities against *et*(M)-carrying strains, the slightly improved activity of TBG-MINO might reflect the slightly better inherent activity noted against *tet*(K)-containing strains. TBG-MINO and DMG-MINO were less active than DMG-DMDOT against *Proteus* spp. and *M. morganii*.

The improved in vitro activity of TBG-MINO was also observed in vivo when its activity against acute lethal infections in mice was tested. When it was dosed intravenously, TBG-MINO was as effective as minocycline against infections caused by minocycline-susceptible bacteria including MRSA and *tet*(K)-containing *S. aureus*. However, the ED_{50}s of TBG-MINO and DMG-DMDOT against infections caused by MRSA that also contained *tet*(M) were lower than those of minocycline. Infections caused by *E. coli* strains carrying *tet*(A), *tet*(B), *tet*(C), or *et*(M) were more responsive to treatment with TBG-MINO or DMG-DMDOT than to treatment with minocycline. The activity of TBG-MINO, however, exceeded the activity of DMG-DMDOT against infections caused by the *tet*(A)- and *tet*(C)-containing strains, thus reflecting the improved in vitro activity of TBG-MINO over that of DMG-DMDOT. Both TBG-MINO and DMG-DMDOT had poor efficacies when they were administered orally.

The ability of TBG-MINO to overcome the major tetracycline resistance mechanisms and extend its spectrum of activity to include multidrug-resistant staphylococci, penicillin-resistant *S. pneumoniae*, vancomycin-resistant enterococci, anaerobes, and minocycline-resistant bacteria while retaining activity against minocycline-susceptible microorganisms makes it an attractive new antibacterial agent. Resistance among *S. pneumoniae*, *Enterococcus* spp., and MRSA is becoming an increasing medical problem worldwide (10, 11, 17, 18, 19, 23, 32), with reduced therapeutic options and an increased need for new antimicrobial agents. TBG-MINO at concentrations of ≤0.5 μg/ml inhibited all strains of penicillin-resistant *S. pneumoniae*, vancomycin-resistant *Enterococcus* spp., and MRSA. Therefore, additional evaluation of TBG-MINO is warranted.

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POLISH JOURNAL OF PHARMACOLOGY AND PHARMACY

VOLUME XXXIV NOVEMBER—DECEMBER FASC. 5—6

ADDRESS OF EDITORIAL OFFICES

Institute of Pharmacology, Polish Academy of Sciences,
Smętna str. 12, phone 77-40-22
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Poland

POLISH ACADEMY OF SCIENCES INSTITUTE OF PHARMACOLOGY
KRAKÓW (POLAND)

POLISH JOURNAL OF PHARMACOLOGY AND PHARMACY

Tom XXXIV

PAŃSTWOWY ZAKŁAD WYDAWNICTW LEKARSKICH

WARSZAWA 1982

Pol. J. Pharmacol. Pharm. 1982, 34, 399—408

KINETICS OF DRUG DECOMPOSITION. PART 73. KINETICS AND MECHANISM OF VITAMIN K_3 SOLUBLE FORM THERMAL DECOMPOSITION IN SOLID PHASE

EWARYST PAWEŁCZYK, BARBARA MARCINIEC

Kinetics of drug decomposition. Part 73. Kinetics and mechanism of vitamin K_3 *soluble form thermal decomposition in solid phase.* E. PAWEŁCZYK, B. MARCINIEC. Pol. J. Pharmacol. Pharm., 1982, 34, 399—408

Kinetics of thermal decomposition of trihydrate of sodium salt of 1,2,3,4-tetrahydro-2-methyl-1,4-dioxo-2-naphtalenesulfonic acid (MDS, vitamin K_3 soluble form) in solid state by accelerated aging method at elevated temperature has been studied. It was found that the process occurs according to Prout-Tompkins model and its rate depends on temperature, humidity and particle size of the substance. Thermodynamic parameters of the reaction (Q_{10}, E_A, $\Delta H^{\neq}$, $\Delta S^{\neq}$, ΔG) were determined and theoretically predicted stability of MDS at room temperature is given.

The reaction mechanism assumes a preliminary dehydration occuring via the successive elimination of one and a half, two and a half and finally three molecules of water. The obtained anhydrous form decomposes thermally forming free radical intermediates and yielding finally 2-methyl-1,4-naphthoquinone (vitamin K_3), SO_2 and NaOH.

Vitamin K_3 or menadione (MD, 2-methyl-1,4-naphthoquinone) is a parent compound for the whole group of vitamins K and their derivatives, however its low water solubility and unstability of solution [4, 6, 10] causes that in the therapy MD is used mainly in solid dosage forms (tablets, dragées). A need for usage of solutions and injections brought about an introduction into therapy the soluble form — menadione sodium hydrosulfite (MDS), which is easily soluble in water (1 : 2).

In the available literature there is a lack of informations on the stability of this compound. This work was aimed at filling up this gap by the investigations on kinetics of thermal decomposition and determination of the mechanism of degradation.

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MATERIAL AND METHODS

Material: Trihydrate of sodium salt of 1,2,3,4-tetrahydro-2-methyl-1,4-dioxo-2-naphthalenesulfonic acid (MDS) produced by Warsaw Pharmaceutical Works "Polfa", batch no. 100676, m.p. 157—158°C, content 99·37% (by ceric salts titration method [5]). Water content assayed by Fischer method [5] was 16·2%. Different fineness of particles was obtained by sieving the substrate through a nylon sieve with the mesh diameter 25, 45 and 80 μm.

Kinetics experiments: Weighed samples (0·0108 g) of the substance in open glass vials were placed in desiccators containing the saturated solution of appropriate inorganic salt to maintain the desired humidity [2]. Then the desiccators were placed in thermal chambers type KBC G-65/250 at the temperatures within 323—353 K. At appropriate time intervals the single vials were withdrawn and the content of non-degraded MDS was assayed by a spectrophotometric method [7] (Fig. 1) which permits to determine MDS in the presence of MD.

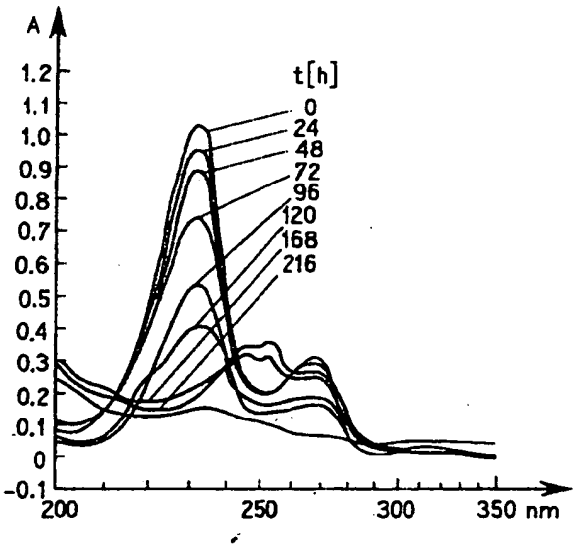

Fig. 1. Charge in UV spectrum the thermal degradation of MDS.

Spectrophotometric assay of MDS: The content of vial was dissolved in 2 ml of methanol and made up with water to 10 ml. This solution after 100-fold dilution served for the measurements of absorbances made in 1 cm absorption cells on Specord UV VIS apparatus at λ_{max} wavelengths 234 and 253 nm. The content of MDS was calculated from appropriate equations [7]. The results obtained by this method have a relative error of 2·7% for MDS and 3·7% for MD.

The kinetic curves were interpreted in terms of the equation for the first order reaction proceeding in accordance with the Prout-Tompkins model [3, 8, 9].

The rate constants of the first order reactions for the acceleration period were calculated from the slope of the linear plot $\lg\frac{x'}{1-x'} = f(t)$, where x′ is a degree of conversion.

The linearity of the plots was estimated by the least squares method.

The water content was determined by Fischer and gravimetric methods [5].

The IR spectra were made in KBr discs (0·5 mg of MDS or MD per 300 mg of KBr) on the Unicam SP 200G apparatus.

The EPR spectra — a sample of 0·1 g of MDS was heated at 368 K (95°C) for 4 h then the EPR spectrum was recorded on the EPR spectrometer Jeol — Co type JESME — 3X (Japan) at 393 K, at the frequency 8·83 GHz.

The DTA, DTG and TG curves were obtained using the Q-Derivatograph apparatus (MOM Budapest) under the following conditions: weighed sample 340 mg, sensitivity 200 mg, temperature increase 5°C/min.

Melting points were determined on Böetius hot stage apparatus (HMK — Dresden).

Chromatography: 0·1 % methanol solutions of the MD standard and the substance isolated from MDS, mp 106—107°C, were applied (0.02 ml) on the plates covered with silica gel HF_{254}. The development was carried out in the solvent system: cyclohexane-chloroform-methanol-acetic acid (2:15:3:1) [1]. In both cases only a single spot, $R_f = 0{\cdot}80$, visualized under the UV light (254 nm) appeared on chromatograms.

Isolation of MD — a weighed sample (3 × 0·1 g) of MDS was heated at 363 K (70°C) until the starting material was completely decomposed (ca. 140 h), then the residue was dissolved in a small water volume, alkalized with ammonia and extracted with $CHCl_3$. The extract was evaporated to dryness under reduced pressure and the residue was crystallized from the mixture water-methanol (100:1), yielding pale yellow needles, mp 106—107°C.

DISCUSSION OF RESULTS

The changes of MDS content in time observed under different conditions of temperature, humidity and fineness of particles are presented in Fig. 2 as plots of degree of conversion vs. time.

The course of the kinetic curves was in all cases S-shaped (sigmoidal) differentiating the induction, acceleration and decline periods of the reaction. The analysis of these curves showed that they follow the first order reaction model proposed by Prout and Tompkins [9].

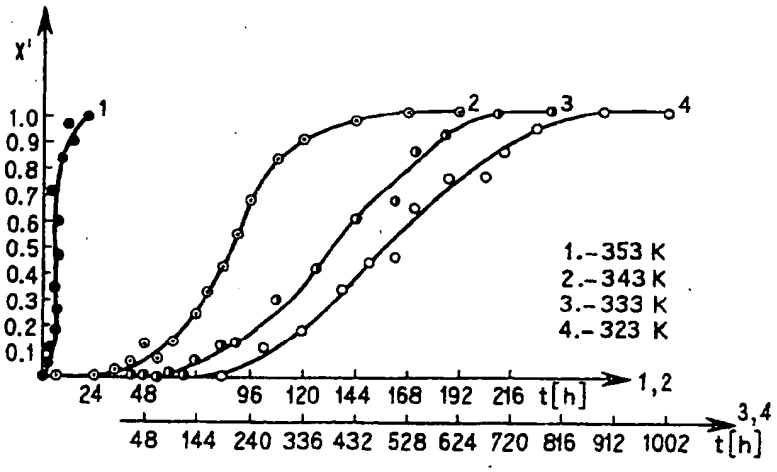

Fig. 2. Dependence of the conversion (x') on time of thermal degradation of MDS.

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Table 1. Comparison of the first-order rate constants for MDS thermal degradation reaction in in the solid state

T (K)	$k \cdot 10^7$ (s^{-1})	Relative humidity %	$k \cdot 10^7$ (s^{-1})	Particle size μm	$k \cdot 10^7$ (s^{-1})
353	2095·7	79·2	223·81	80	178·87
343	193·13	61·7	193·13	45	155·49
333	35·853	50·7	176·20	25	105·22
323	21·333	for temperature 343 K and particle size 80 μm		for temperature 343 K and relative humidity 61·7%	
for relative humidity 61·7% and particle size 80 μm					

A comparison of the observed first order rate constants for the acceleration period (Table 1) clearly demonstrates that the temperature has the greatest effect on the rate of the reaction. It also determines the magnitude of the induction period (Fig. 3), which is inversely proportional to the temperature according to the function $I = f(T)$, where I is the time of the induction period. In the range of the investigated temperatures (323—353 K) the factor Q_{10} is not constant but increases from the value 1·68 for the temperature range 323—333 K to the value 10·85 for the range 343—353 K. This is reflected in the slope of the Arrhenius plot $\log k = f\left(\frac{1}{T}\right)$ (Fig. 4) and affects the values of thermodynamic parameters and the stability of MDS at room temperature (Table 2).

From Table 2 it follows that the time values $t_{10\%}$ are quite different, when calculated for the acceleration period and for the reaction when the induction period is taken into account. Thus, the time in which 10% of the starting material is decomposed amounts 267 days for the acceleration period at room temperature, while taking into account the induction period its value is ca. 14 years (Table 2, Fig. 3).

Besides the temperature also the humidity and the particle size have an influence upon the stability of MDS, althought this effects is not as significant as in the case of the temperature. The values of the first order rate constants calculated for the acceleration period increase proportionally to the increase of percentage of relative humidity but this increase is quite small (Fig. 5). In general, the following regularity holds: 10% increase in the humidity causes 10% acceleration of the degradation process (Table 1, Fig. 5).

The size of particles of the substance has somewhat greater effect on the rate of degradation of MDS. At constant humidity the rate constants increase proportionally to the diameter of particles (Table 1, Fig. 5), what was confirmed in several times repeated determinations at different temperatures.

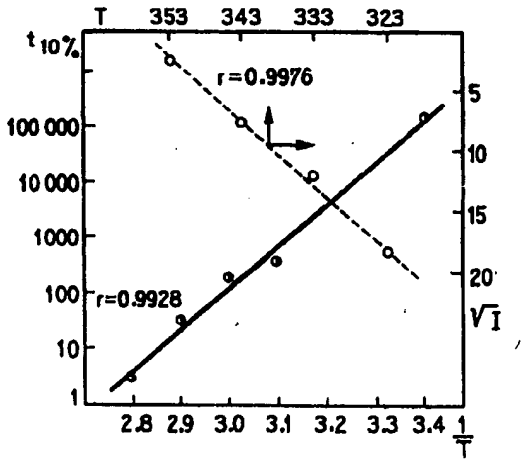

Fig. 3. Square root of the induction period $\sqrt{I}$ vs. temperature and $t_{10\%}$ time (calculated from the Prout-Tompkins' equation) vs. the reciprocal of the temperature $\left(\frac{1}{T}\right)$.

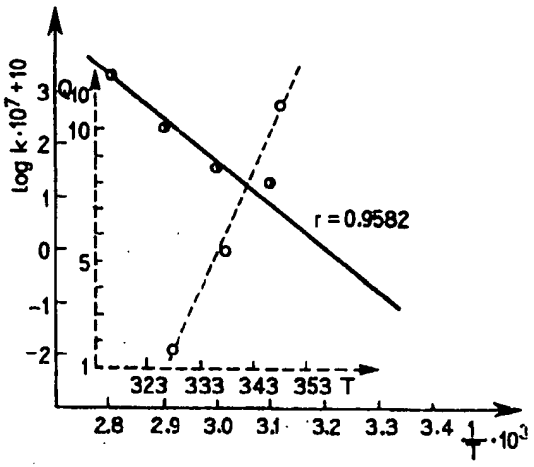

Fig. 4. Arrhenius' plot for thermal degradation of MDS (——) and the dependence of the temperature coefficients (Q_{10}) on temperature (- - -).

Table 2. Kinetic and thermodynamic parameters for MDS desulfonation reaction in the solid state (relative humidity 61·7%, particle size 80 μm)

T (K)	k·10⁷ (s^{-1})	Q_{10}	$t_{10\%}$	$t_{10\%}$*)	E_A (KJ·mol⁻¹)	$\Delta H^{\neq}$ (KJ·mol⁻¹)	$\Delta S^{\neq}$ (KJ·K⁻¹·mol⁻¹)	ΔG (KJ·mol⁻¹)
353	2095·664	10·85	8·3 min	2·9 h			−128·58	98·118
343	193·128	5·38	1·5 h	31·5 h			−130·85	98·625
333	35·853	1·38	8·2 h	169·1 h	146·475	143·506	−144·47	92·751
323	21·333		13·7 h	280·3 h			−157·14	92·168
293	0·046		267·3 days	14 years				

*) with regard to induction period

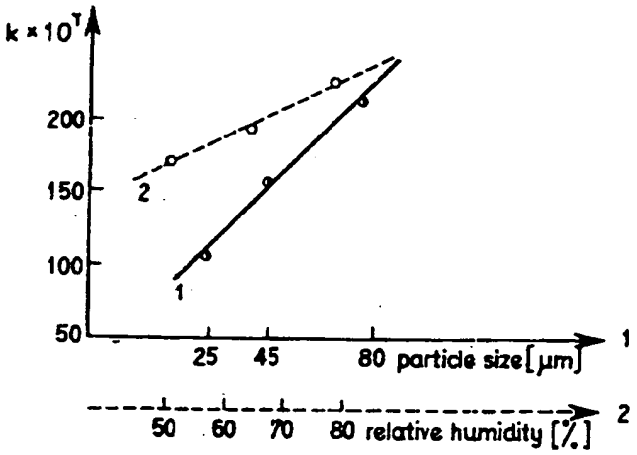

Fig. 5. Dependence of the rate constant (k) of thermal degradation of MDS on the relative humidity (—) and on particle size (- - -).

This relationship and the acceleration of reaction for the higher particle sizes is contradictory to general rules of the kinetics of reactions in the solid state, but this contradiction is only apparent. The general opinion is that a decrease of the particles diameter is associated with an increase of the degradation rate of the solid phase of the substance. In the majority of cases it is true, since the decrease of the particles diameter results in an increase of the area where the specific degradation reaction takes place. Because these reactions are mostly hydrolysis and oxidation reactions there are no deviations from this rule. It is however different when gaseous products are formed during the degradation process, what is the case in the degradation of MDS (Fig. 6, Table 3). Then the rate of this reaction depends mainly upon the rate of gas diffusion in the degraded substance. It even seems that in the case of decreased area and thus the greater particle sizes (but at the same time the greater free space between these particles) the diffusion would be facilitated and its rate enhanced, what is manifested in the acceleration of the degradation process as observed in our experiments.

The differential thermal analysis: DTA and DTG of the MDS sample (Fig. 6) showed, that the degradation occurs as a result of the consecutive endothermic (peaks A and B) and one exothermic (peak C) reactions accompanied by appropriate losses of the sample weight. The following successive weight losses were found from the TG curve: 8·21%, 13·26% and 35·57%. They correspond with the elimination of one and a half of water molecule at 105°C, two and a half water molecules at 147°C and three water molecules and a molecule of SO_2 at 230°C (Fig. 6, Table 3). Thus, the elimination of water, i.e. the gradual dehydration of the MDS molecule is the endothermic reaction, while the desulfonation itself is exothermic.

A similar mechanism of degradation at lower temperatures under isothermal conditions is suggested by the results in Table 3, where one can see that during the induction period the content of MDS stepwise decreases by ca. 5,11 and 16%, what corresponds with the loss of one, two and three molecules of water, respectively. During the further degradation process a loss of weight of the substrate is also observed.

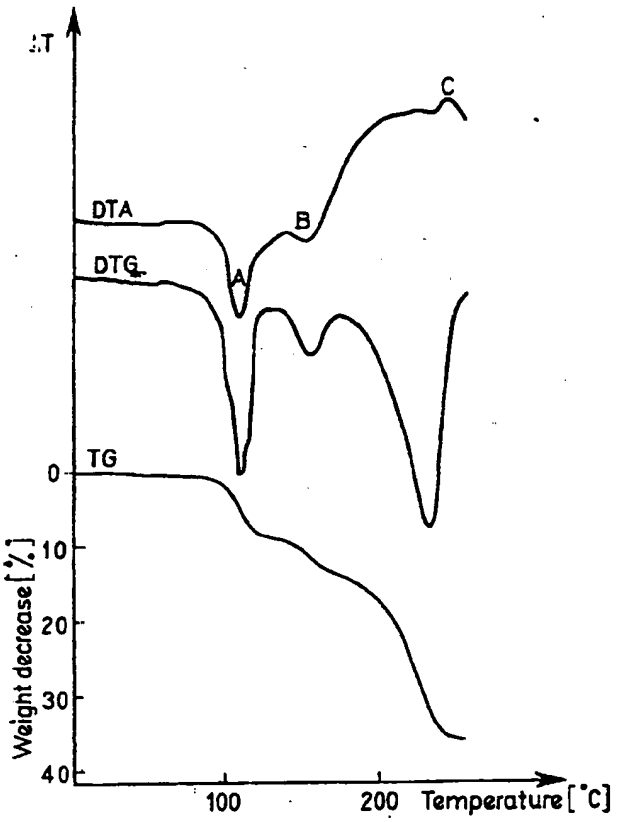

Fig. 6. DTA, DTG and TG curves of MDS.

The further thermal degradation of MDS monitored by UV, EPR and IR spectra (Fig. 7—9) proceeds as follows: in the first step of degradation, i.e. dehydration, the UV spectrum remains qualitatively unchanged with only minor quantitative changes. Then, when the sample changes its coloration to rose-red-violet, a significant batochromic shift occurs in the UV spectrum for both maxima at λ_{234}nm and λ_{330}nm (Fig. 7).

This step of degradation corresponds to the formation of free radicals (MDS) * of chinoid and semichinoid type, what was confirmed by the EPR spectrum (Fig. 8).

Table 3. Compliation of data for water determination during the induction period of thermal degradation of MDS

T (K)	t (h)	Fischer method		Grawimetric method	
		water concentration (%)	MDS decrease (%)	water concentration (%)	MDS decrease (%)
	3	10·23	6·11	11·37	4·98
343	6	6·32	10·03	4·87	11·48
	20	0·51	15·84	0·32	16·03
	20	10·32	6·13	10·32	6·03
323	48	3·35	13·00	4·87	11·48
	120	0	16·64	0·15	16·20

*) the weight decrease was found also in subsequent stages of the reaction

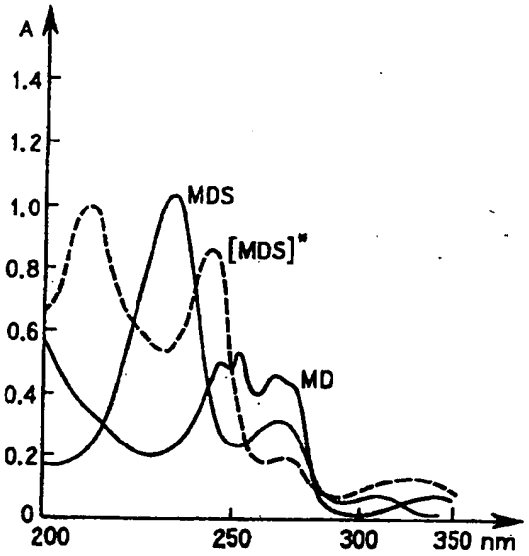

Fig. 7. UV spectrum of MDS, MD and free radicals forming during thermal degradation of MDS (solvent: methanol — water; 3 : 1).

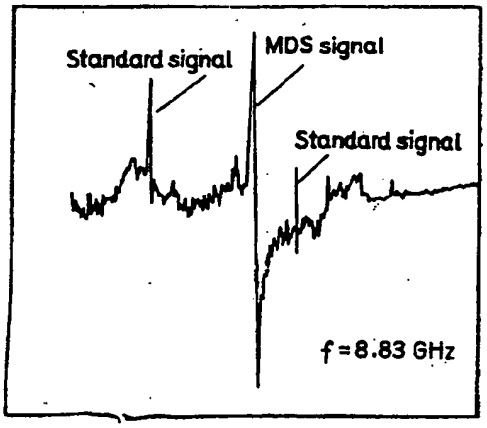

Fig. 8. EPR (ESR) spectrum of MDS in solid state, heated at 368 K within 4 h.

The spectroscopic splitting factor (Landé factor) g was calculated from the expression:

$$E = h \cdot r = g \cdot \mu_B \cdot H$$

where H = magnetic field strenght, μ_B = Bohr magneton, h = Planck constant and r = EPR radiospectroscopic density. The obtained value $g \approx 2{\cdot}8 \times 10^{-5}$, proves, that the signal should be attributed to the unpaired, localized electron.

In the next stage of degradation new maxima were observed in the UV spectrum in 240—350 nm region. Their location and shape are in agreement with spectrum of the MD standard, what demonstrates that MD is a final product of degradation of MDS. This was also proved by chromatographic and IR analysis of the substance isolated from the decomposed MDS sample. This substance was chromatographically homogenous, forming pale yellow needles, mp 106—107°C, and has the IR spectrum identical with that of the MD standard (Fig. 9).

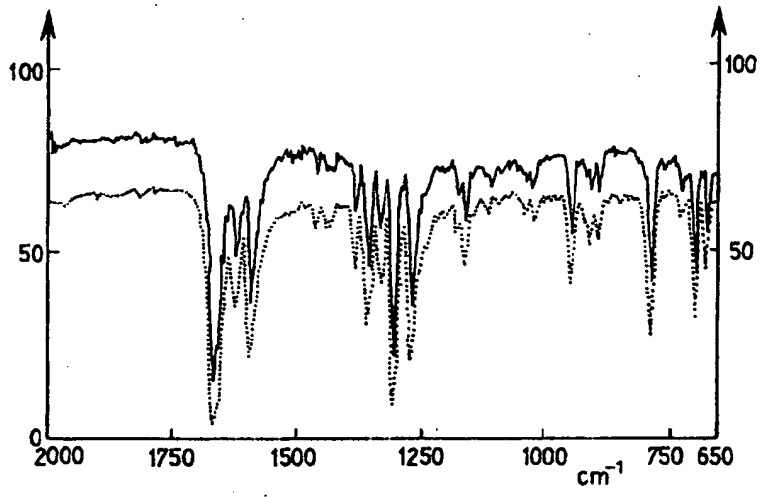

Fig. 9. IR spectrum of MD. ——standard sample; — — — the compound isolated during thermal degradation of MDS.

In conclusion, the thermal degradation of MDS both at increasing temperature and at constant (elevated) temperature is a three-step consecutive reaction with the following steps: I — dehydration, II — formation of free radicals, III — specific desulfonation, what can be represented by the scheme:

$$\text{MDS} \cdot 3H_2O \xrightarrow{\text{I}} \text{MDS} \cdot 1\tfrac{1}{2}\,H_2O \xrightarrow{\text{I}} \text{MDS} \cdot \tfrac{1}{2}\,H_2O \xrightarrow{\text{I}}$$
$$\text{MDS} \xrightarrow{\text{II}} (\text{MDS})^* \xrightarrow{\text{III}} \text{MD} + SO_2 + \text{NaOH}$$

or in shorter form:

$$\text{MDS} \cdot 3\,H_2O \xrightarrow{\text{I}} \text{MDS} \xrightarrow{\text{II}} (\text{MDS})^* \xrightarrow{\text{III}} \text{MD} + SO_2 + \text{NaOH}$$

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Received: 19 January 1981.
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VOLUME 44, NUMBER 8 PUBLISHED MONTHLY AUGUST 2000

ANTIMICROBIAL AGENTS AND CHEMOTHERAPY

AMERICAN SOCIETY FOR MICROBIOLOGY

ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Aug. 2000, p. 2225–2229 Vol. 44, No. 8
0066-4804/00/$04.00+0

In Vitro Activities of the Glycylcycline GAR-936 against Gram-Positive Bacteria

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Received 1 February 2000/Returned for modification 18 April 2000/Accepted 2 May 2000

The in vitro activities of GAR-936, the 9-*t*-butylglycylamido derivative of minocycline, were compared with those of doxycycline, minocycline, and tetracycline against 527 gram-positive clinical isolates. GAR-936 inhibited all strains, including those resistant to other tetracyclines, at concentrations of ≤2 μg/ml, except two strains of JK diphtheroids for which the MIC was 4 μg/ml.

Although tetracyclines remain valuable therapeutic agents for a variety of infections, resistance to this class limits their use against many important gram-positive bacterial pathogens. For example, only 33% of enterococci recovered at our hospital during 1997 to 1999 were susceptible to tetracycline. The glycylcyclines are novel tetracycline analogs that have activity against organisms resistant to older compounds of this class (5, 14). They inhibit protein synthesis on wild-type ribosomes and on TetM-protected, tetracycline-resistant ribosomes (15). These compounds also inhibit organisms with tetracycline efflux determinants (14). GAR-936, the 9-*t*-butylglycylamido derivative of minocycline, appears to be both better tolerated by hosts and more active against tetracycline-resistant strains than earlier glycylcyclines (14). The present study examined the in vitro activities of GAR-936 against gram-positive bacteria, including strains resistant to β-lactams, glycopeptides, and other tetracyclines.

Routine clinical isolates collected at the Beth Israel Deaconess Medical Center were included regardless of tetracycline resistance patterns. Staphylococci and most pneumococci were recovered in 1998. Strains with unusual resistance traits, including glycopeptide-resistant or β-lactamase-producing enterococci and β-lactam-resistant streptococci, had been referred to our laboratory from various sources over several years (2, 4, 16). Wyeth-Ayerst Laboratories, Pearl River, N.Y., kindly provided GAR-936. Tetracycline, minocycline, and doxycycline were purchased from Sigma Chemical Company, St. Louis, Mo.

Activities of the antimicrobials were determined by agar dilution methods on Mueller-Hinton II agar (12). Media were supplemented with 5% sheep blood for testing streptococci and corynebacteria. Inocula of approximately 10^4 CFU were applied to the surfaces of plates and were incubated for 16 to 20 h at 35°C in air or in 5% CO_2 (for *Lactobacillus* spp., *Leuconostoc* spp., *Streptococcus* spp., *Pediococcus* spp., and diphtheroids). Pneumococci were also tested by broth microdilution (12).

The results of susceptibility studies are shown in Table 1. GAR-936 was four times less active than minocycline against oxacillin-susceptible and -resistant strains of *Staphylococcus aureus*, according to a comparison of MICs at which 90% of the isolates tested were inhibited (MIC_{90}s). However, isolates intermediately susceptible or resistant to the other compounds were inhibited by GAR-936 at ≤1 μg/ml. GAR-936 inhibited two strains of vancomycin-intermediate *S. aureus* (resistant to tetracycline and minocycline) at concentrations of 1 and 2 μg/ml.

Against coagulase-negative staphylococci, GAR-936 was consistently 1 dilution less active than minocycline, based on the MIC_{50}s and MIC_{90}s. Again, however, the new compound inhibited tetracycline- and doxycycline-resistant strains at ≤2 μg/ml. The MICs of GAR-936 for staphylococci resistant to both tetracycline and minocycline were higher than those for strains susceptible to minocycline (Fig. 1).

Against streptococci other than pneumococci, the intrinsic potency of GAR-936, based on a comparison of MIC_{50}s, was equivalent or slightly superior to that of minocycline. Median (modal) MICs for GAR-936 and minocycline were 0.06 (0.06) and 0.12 (0.12) μg/ml, respectively. However, all streptococcal isolates, including strains resistant to doxycycline or minocycline, were inhibited by GAR-936 at ≤0.25 μg/ml.

For pneumococci, the results shown in Table 1 were obtained by agar dilution. The activity of GAR-936 against 60 strains of *Streptococcus pneumoniae*, half of which were not susceptible to penicillin, was also evaluated by microdilution. The MIC_{50} and MIC_{90} were 0.03 and 0.06 μg/ml. These were within 1 dilution of agar dilution results. Individually, microdilution results were 2 dilutions lower than the agar dilution results for 11 strains, 1 dilution lower for 27, equivalent for 13, and 1 dilution greater for 4. For *S. pneumoniae* ATCC 49619, the broth microdilution result was 1 dilution lower than the agar dilution result.

Approximately one-third of enterococci were resistant to doxycycline or minocycline and 44% were resistant to tetracycline, whereas all were inhibited by GAR-936 at ≤2 μg/ml. Against 20 strains of *Listeria monocytogenes*, GAR-936 was equal in activity to minocycline and doxycycline, based on a comparison of MIC_{50}s, but eight times more active than tetracycline. GAR-936 was the most active agent tested against *Lactobacillus*, *Pediococcus*, and *Leuconostoc*. Against *Pediococcus* spp., GAR-936 was up to 4 times more active than minocycline and 8 to 128 times more active against doxycycline and tetracycline, based on a comparison of MIC_{90}s. The isolates least susceptible to the new drug were *Corynebacterium jeikeium*. Two isolates of this group were inhibited only at 4 μg/ml.

Figure 1 shows the mean MICs of GAR-936 for isolates classified by susceptibility to tetracycline and minocycline. The geometric mean MIC of GAR-936 for tetracycline-resistant,

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TABLE 1. Comparative in vitro activity of GAR-936

Organism(s) and relevant characteristic (no. of isolates)	Antimicrobial agent	MIC (μg/ml)		
		Range	50%	90%
Enterococcus faecalis (42)	GAR-936	0.06–0.5	0.12	0.25
	Minocycline	0.06–32	16	32
	Doxycycline	0.12–32	8	16
	Tetracycline	0.12–>128	32	128
Enterococcus faecalis, β-lactamase producing (10)	GAR-936	0.12–0.25	0.25	0.25
	Minocycline	8–16	16	16
	Doxycycline	8–16	8	16
	Tetracycline	32–>128	32	64
Enterococcus faecalis, vancomycin resistant (VanA) (10)	GAR-936	0.12–1	0.25	0.5
	Minocycline	0.12–32	0.12	16
	Doxycycline	0.25–16	0.25	16
	Tetracycline	0.5–>128	0.5	64
Enterococcus faecalis, vancomycin resistant (VanB) (20)	GAR-936	0.12–0.25	0.25	0.25
	Minocycline	0.12–32	0.25	16
	Doxycycline	0.12–32	0.5	32
	Tetracycline	0.03–>128	1	>128
Enterococcus faecium (40)	GAR-936	0.06–0.25	0.12	0.25
	Minocycline	0.06–64	0.06	16
	Doxycycline	0.12–64	0.12	16
	Tetracycline	0.12–>128	0.25	64
Enterococcus faecium, vancomycin resistant (VanA) (24)	GAR-936	0.06–0.25	0.12	0.25
	Minocycline	0.06–32	0.12	16
	Doxycycline	0.12–32	0.12	16
	Tetracycline	0.03–128	0.25	64
Enterococcus faecium, vancomycin resistant (VanB) (20)	GAR-936	0.12–0.5	0.25	0.25
	Minocycline	0.06–32	16	32
	Doxycycline	0.12–32	8	32
	Tetracycline	0.12–128	32	64
Enterococcus faecium, vancomycin resistant (VanD) (4)	GAR-936	0.06		
	Minocycline	0.06–2		
	Doxycycline	0.12–4		
	Tetracycline	0.25–16		
Enterococcus avium (10)	GAR-936	0.06–0.12	0.06	0.06
	Minocycline	0.06–16	8	16
	Doxycycline	0.25–32	8	32
	Tetracycline	0.5–>128	32	64
Enterococcus raffinosus (10)	GAR-936	0.06–0.5	0.06	0.12
	Minocycline	0.06–32	0.06	16
	Doxycycline	0.12–16	0.25	16
	Tetracycline	0.25–128	0.5	64
Enterococcus casseliflavus (14)	GAR-936	0.12–0.5	0.25	0.25
	Minocycline	0.06–0.12	0.06	0.12
	Doxycycline	0.12–0.25	0.25	0.25
	Tetracycline	1–2	2	2
Enterococcus gallinarum (11)	GAR-936	0.06–2	0.12	0.25
	Minocycline	0.06–32	0.12	16
	Doxycycline	0.12–16	0.25	16
	Tetracycline	0.25–128	1	128
Staphylococcus aureus, oxacillin susceptible (30)	GAR-936	0.25–0.5	0.5	0.5
	Minocycline	0.06–8	0.12	0.12
	Doxycycline	0.25–8	0.25	0.5
	Tetracycline	0.12–64	0.5	1
Staphylococcus aureus, oxacillin resistant (28)	GAR-936	0.25–1	0.5	1
	Minocycline	0.06–8	0.25	0.25
	Doxycycline	0.12–16	0.25	2
	Tetracycline	0.25–64	0.5	1

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TABLE 1—*Continued*

Organism(s) and relevant characteristic (no. of isolates)	Antimicrobial agent	MIC (μg/ml)		
		Range	50%	90%
Staphylococcus aureus, vancomycin intermediate (2)	GAR-936	1–2		
	Minocycline	16		
	Doxycycline	8		
	Tetracycline	64		
Coagulase-negative staphylococci, oxacillin susceptible (24)	GAR-936	0.12–1	0.25	1
	Minocycline	0.12–1	0.12	0.5
	Doxycycline	0.12–64	0.25	2
	Tetracycline	0.25–>128	0.5	4
Coagulase-negative staphylococci, oxacillin resistant (30)	GAR-936	0.12–2	0.5	1
	Minocycline	0.06–2	0.25	0.5
	Doxycycline	0.12–32	1	8
	Tetracycline	0.25–>128	2	64
Streptococcus pyogenes (30)	GAR-936	0.06–0.25	0.12	0.12
	Minocycline	0.12–16	0.12	0.25
	Doxycycline	0.25–16	0.25	0.5
	Tetracycline	0.25–32	0.5	4
Streptococcus agalactiae (10)	GAR-936	0.06–0.25	0.12	0.12
	Minocycline	0.12–32	16	32
	Doxycycline	0.25–16	16	16
	Tetracycline	0.5–64	64	64
Viridans group streptococci, penicillin susceptible (15)	GAR-936	0.03–0.25	0.06	0.25
	Minocycline	0.03–4	0.12	2
	Doxycycline	0.06–8	0.25	4
	Tetracycline	0.03–8	0.25	8
Viridans group streptococci, penicillin resistant (15)	GAR-936	0.02–0.12	0.03	0.06
	Minocycline	0.06–32	0.12	16
	Doxycycline	0.02–32	0.25	16
	Tetracycline	0.02–64	0.25	64
Streptococcus pneumoniae, penicillin susceptible (28)	GAR-936	0.03–0.25	0.06	0.12
	Minocycline	0.06–16	0.06	8
	Doxycycline	0.12–16	0.25	16
	Tetracycline	0.12–64	0.25	32
Streptococcus pneumoniae, penicillin resistant (30)	GAR-936	0.03–0.25	0.06	0.12
	Minocycline	0.06–16	0.12	16
	Doxycycline	0.06–32	0.25	16
	Tetracycline	0.12–64	0.25	32
Listeria monocytogenes (20)	GAR-936	0.25–0.5	0.25	0.5
	Minocycline	0.12–0.25	0.25	0.25
	Doxycycline	0.12–0.25	0.25	0.25
	Tetracycline	0.5–4	4	4
JK diphtheroids (20)	GAR-936	0.12–4	0.5	2
	Minocycline	0.12–4	0.25	1
	Doxycycline	0.25–8	0.25	4
	Tetracycline	0.25–64	0.5	32
Lactobacillus spp. (12)	GAR-936	0.03–0.12	0.06	0.12
	Minocycline	0.03–2	0.12	0.5
	Doxycycline	0.12–8	0.25	2
	Tetracycline	0.12–8	0.5	8
Leuconostoc spp. (10)	GAR-936	0.12	0.12	0.12
	Minocycline	0.25–0.5	0.25	0.5
	Doxycycline	1–4	1	2
	Tetracycline	1–8	1	4
Pediococcus spp. (8)	GAR-936	0.03–1		
	Minocycline	0.5–2		
	Doxycycline	2–8		
	Tetracycline	4–128		